AUTOPSY BASED STUDY OF HISTOPATHOLOGY AND BIOCHEMICAL MARKERS OF THYROID GLAND IN CASES OF HANGING.

Thesis

Submitted to the faculty of

ALL INDIA INSTITUTE OF MEDICAL SCIENCES, BHOPAL

For the award of the degree of

Doctor of Medicine (MD)

in

Forensic Medicine and Toxicology

by

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Abbreviations

HIV	HUMAN IMMUNODEFICIENCY VIRUS
AIDS-	ACQUIRED IMMUNODEFICIENCY SYNDROME
TG	THYROGLOBULIN
T4	THYROXINE
T3	TRIIODOTHYRONINE
FT3	FREE T3
TT4	TOTAL T4
TSH	THYROID-STIMULATING HORMONE
C5- T1	CERVICAL5 - THORACIC1 VERTEBRA
TRH	THYROTROPIN-RELEASING HORMONE (
WHO	WORLD HEALTH ORGANIZATION
5-HT	5-HYDROXYTRYPTAMINE
SIDS	SUDDEN INFANT DEATH SYNDROME
RT3	REVERSE T3
ELISA	ENZYME-LINKED IMMUNOSORBENT ASSAY
CSF	CEREBROSPINAL FLUID
FT4	FREE T4
PTH	PARATHORMONE
AIIMS	ALL INDIA INSTITUTE OF MEDICAL SCIENCES
HCL	HYDROCHLORIC ACID
H&E	HEMATOXYLIN AND EOSIN
ROC	RECEIVER OPERATING CHARACTERISTIC

CHAPTER 1: INTRODUCTION

INTRODUCTION

Suicide can be considered as a premature ending of life whose ripples affect the lives of their loved ones. Each year, 703,000 people die by suicide worldwide. It ranks as one of the leading causes of death globally, surpassing deaths from malaria, HIV/AIDS, breast cancer, war, and homicide. In 2019 according to WHO, more than 1 in every 100 deaths (1.3%) was due to suicide. (1)

Recent studies indicate changing patterns in suicide methods and rates in India (2). With a higher female-to-male ratio and with marital status not necessarily protective India's suicide patterns differ from global trends (3). Hanging is a prevalent method of suicide in India, second only to poisoning (4). Hanging is a type of strangulation characterized by occlusion of the blood vessels and air passages of the neck because of external pressure that leads to cerebral hypoxia (5). The force applied to the neck is the gravitational drag of part or whole weight of the body (6). People who opted for or thought about hanging as a method of suicide viewed it as a sure, swift, and "clean" way to die, with limited understanding of the actual dying process. The ease of access and perceived simplicity of hanging were significant reasons for its choice, as respondents noted that the necessary materials were easily obtainable, and they believed it required minimal planning or technical skill(7).

The discipline of forensic medicine, also known as legal medicine, involves the application of medical knowledge to legal issues and investigations. This field is crucial in determining the cause of injuries or death, which can be pivotal in both criminal and civil cases. Forensic experts meticulously analyze findings such as autopsy results, toxicology reports, and injury patterns to establish correlations between observed medical conditions and potential causes. This process is vital for reconstructing events leading to an injury or death, thereby providing critical evidence in court.

In some cases, such as hangings, the external and internal signs may be subtle or even absent, making it challenging to determine the cause of death through traditional methods. Additionally, crime scene examinations may yield no results, or there may be no witnesses to provide information. In these situations, other investigative techniques become essential. Postmortem biochemistry can help detect chemical changes in the body that occur after death, while histopathology involves the microscopic examination of tissues to identify disease or injury. These methods can provide additional evidence to support the findings of the forensic

investigation, ensuring a comprehensive understanding of the circumstances surrounding the death.

In such cases, investigating changes in structures like the skin, soft tissues, glands (such as salivary, thyroid, and parathyroid), lymph nodes, and blood vessels can be very informative. Due to its location in the neck and relatively large size, the thyroid gland is particularly likely to be affected. Numerous studies have examined post-mortem human body fluids in hanging cases, focusing significantly on thyroglobulin levels. Thyroglobulin(Tg), stored in colloid form within the thyroid gland, becomes a key marker. The destruction of colloid-containing follicles, similar histopathological changes, and increased thyroglobulin levels in blood samples from sites distant from the thyroid gland can indicate a vital reaction.(8).

In forensic pathology, distinguishing between antemortem and postmortem injuries is based on the presence of vital reactions. This distinction is crucial as it helps determine whether the person was alive at the time of hanging, thereby strengthening the diagnosis. By analysing these changes, forensic experts can gather critical evidence to support their findings and provide a better understanding of the circumstances surrounding the death.

Similarly, thyroid hormones, including thyroxine (T4) and triiodothyronine (T3), are vital for regulating the body's metabolism. Produced by the thyroid gland, these hormones influence how the body uses energy, affecting key processes such as weight management, heart rate, and body temperature. T3, the more active form, is converted from T4 in various tissues and directly impacts cellular functions, ensuring that cells operate efficiently. Beyond their metabolic roles, thyroid hormones significantly affect the brain's neurotransmitters, including serotonin, dopamine, and norepinephrine. These neurotransmitters are crucial for mood regulation and cognitive processes. When thyroid hormone levels are imbalanced, it can lead to various mental health issues. For instance, hypothyroidism, characterized by low levels of thyroid hormones, is often associated with symptoms like depression, fatigue, and cognitive impairment. This is because insufficient thyroid hormones can slow down brain function and reduce the production of neurotransmitters that regulate mood. Conversely, hyperthyroidism, where there is an excess of thyroid hormones, can lead to anxiety, irritability, and mood swings. The overproduction of thyroid hormones can overstimulate the brain, leading to heightened anxiety and irritability. This imbalance can also disrupt sleep patterns and cause rapid heart rates, further contributing to mood disturbances.

Several studies have investigated the relationship between thyroid function and suicidal behaviour. Lower levels of Free T3(FT3) and Total T4(TT4) were found in individuals with suicidal behaviour compared to controls(9). This decrease in FT3 levels was comparatively more pronounced in female suicidal patients as compared to male suicidal patients(10). Some studies reported lower TSH levels in suicide attempters, and individuals with subclinical hyperthyroidism had more suicide attempts than the control group(11).

Therefore, in order to better understand the role of thyroid hormones, locally stored thyroglobulin and the changes in thyroid gland cases of hanging with various circumstances and accessible case history can be taken into account and compared with other non-hanging cases. The outcome of this study will be able to help and provide forensic experts, clinical psychologists/psychiatrists, and law enforcement officials with scientific explanations regarding the changes in the thyroid gland and associated hormonal and chemical alterations. This information could be crucial in determining the presence of suicidal ideation, confirming hanging as the cause of death, and establishing whether the hanging occurred before or after death.

Incorporating this knowledge into investigations and clinical practice can help distinguish between evidence of foul play and natural alterations in cases of death. Additionally, it can improve the care and treatment of patients experiencing similar conditions, ensuring they receive appropriate support and intervention.

CHAPTER 2: REVIEW OF LITERATURE

REVIEW OF LITERATURE

THYROID GLAND

The thyroid gland, the body's largest endocrine gland, is situated in the front of the neck at the C5- T1 vertebral level and consists of two lobes interconnected by an isthmus in the midline surrounded by a connective tissue capsule to form a butterfly-like shape (Figure 1)(12). It plays a crucial role in regulating basal metabolism and various physiological functions. The parathyroid glands, which are embedded within the thyroid, help maintain calcium balance in the body(13). The average weight of the thyroid gland was 19.9 grams in males and 13.9 grams in females. Additionally, there is a significant negative correlation between age and thyroid size(14).

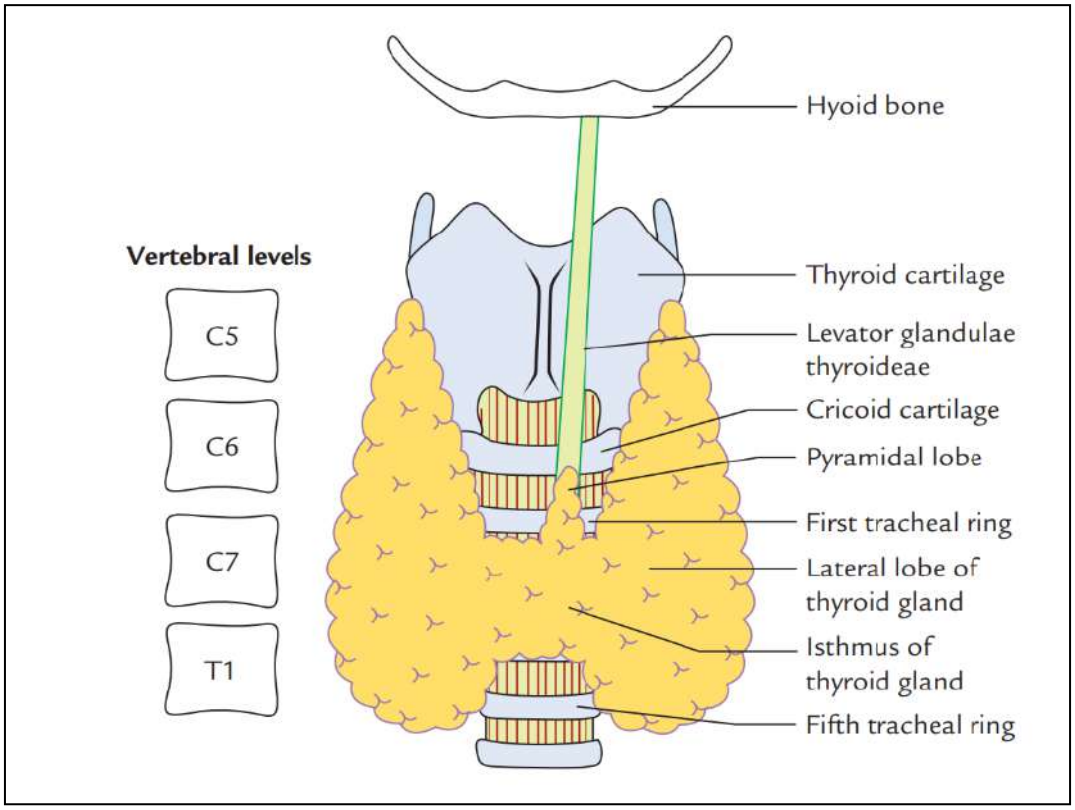


Figure 1: Parts and extend of thyroid gland (12)

The inferior and superior thyroid arteries mainly supply the thyroid gland, and it features a complex network of blood capillaries surrounding each follicle, which ultimately drains through the superior, middle, or inferior thyroid veins(15,16).

Histologically, the thyroid gland consists of follicles lined with cuboidal epithelial cells and parafollicular cells (C-cells). Follicular cells are responsible for the production of thyroid hormones. The thyroid follicles have a lumen that stores thyroglobulin, a glycoprotein precursor

to T3 and T4. This thyroglobulin is iodinated, reabsorbed, hydrolyzed, and then released as the thyroid hormones T3 and T4. The parafollicular cells, also known as C-cells, secrete the hormone calcitonin(17). Like other endocrine glands, the thyroid gland is regulated by a feedback system involving the hypothalamus, pituitary, and thyroid. The hypothalamus produces thyrotropin-releasing hormone (TRH), secreted into the venous system, that drains to the pituitary gland. TRH binds to receptors in thyrotroph cells at the pituitary, leading to the production and secretion of thyroid-stimulating hormone (TSH), also known as thyrotropin. TSH then binds to receptors in the thyroid gland's follicular cells, stimulating the production and release of the thyroid hormones T3 and T4(18).

Histology

The structure is divided into lobules containing 20 to 40 round to oval follicles, each measuring 50 to 500 microns. These follicles are lined with a single layer of cuboidal to low columnar epithelium (Figure 2). The lumen contains colloid, which appears scalloped and pale in follicles with active secretory activity, densely eosinophilic in inactive follicles, and more flocculent and basophilic in elderly individuals. Occasionally, clusters of small follicles that extend into the lumen of larger, actively secreting follicles are present, known as Sanderson polsters(19).

Figure 2:Thyroid follicular epithelium: flat, cuboidal and columnar (H&E, high power) (19).

Histopathology of common thyroid pathologies.

Graves Disease involves autoantibodies that target the TSH receptors on follicular cells, leading to increased thyroid hormone production. This condition results in severely hyperplastic follicles with papillae extending into them. These follicles contain pale colloid with characteristic scalloped edges(20).

Hashimoto Thyroiditis is an autoimmune disorder where the body's immune system attacks the thyroid gland. The presence of antithyroglobulin and antithyroid peroxidase antibodies characterizes this condition. In this condition, the interstitium is infiltrated by lymphocytes, forming reactive follicles. Additionally, there is Hürthle cell metaplasia of the follicular epithelium(20).

SUICIDE

Epidemiology of suicide

Globally, the age-standardized suicide rate is significantly higher in males (12.6 per 100,000) than in females (5.4 per 100,000). While female rates in some countries exceed 10 per 100,000, male rates exceed 45 per 100,000. Overall, the male suicide rate is 2.3 times higher than the female rate. In high-income countries, the male-to-female (M: F) suicide ratio is slightly over 3, whereas it is lower in low- and middle-income countries (2.9 in low-income, 1.8 in lower-middle-income, and 2.6 in upper-middle-income countries(Figure3).(1)

In her 2018 review, Silke Bachmann conducted a comprehensive analysis based on a literature search of the World Health Organization (WHO) databases and PubMed. The review highlights that globally, suicides are the second leading cause of premature mortality among individuals aged 15 to 29 years, following traffic accidents, and the third leading cause in the 15 to 44 years age group(21,22). According to the WHO, children and adolescents up to the age of 15 exhibit the lowest global suicide rates, which steadily increase until the age of 70 and above(23). The review also notes excessively high suicide rates among young adults in low-income countries. The overall suicide rate stands at 10.7 per 100,000 population, with a male-to-female ratio of 1.7. A ratio below one indicates that the suicide rate among females surpasses that of males, a phenomenon observed only in China and Bangladesh(22).

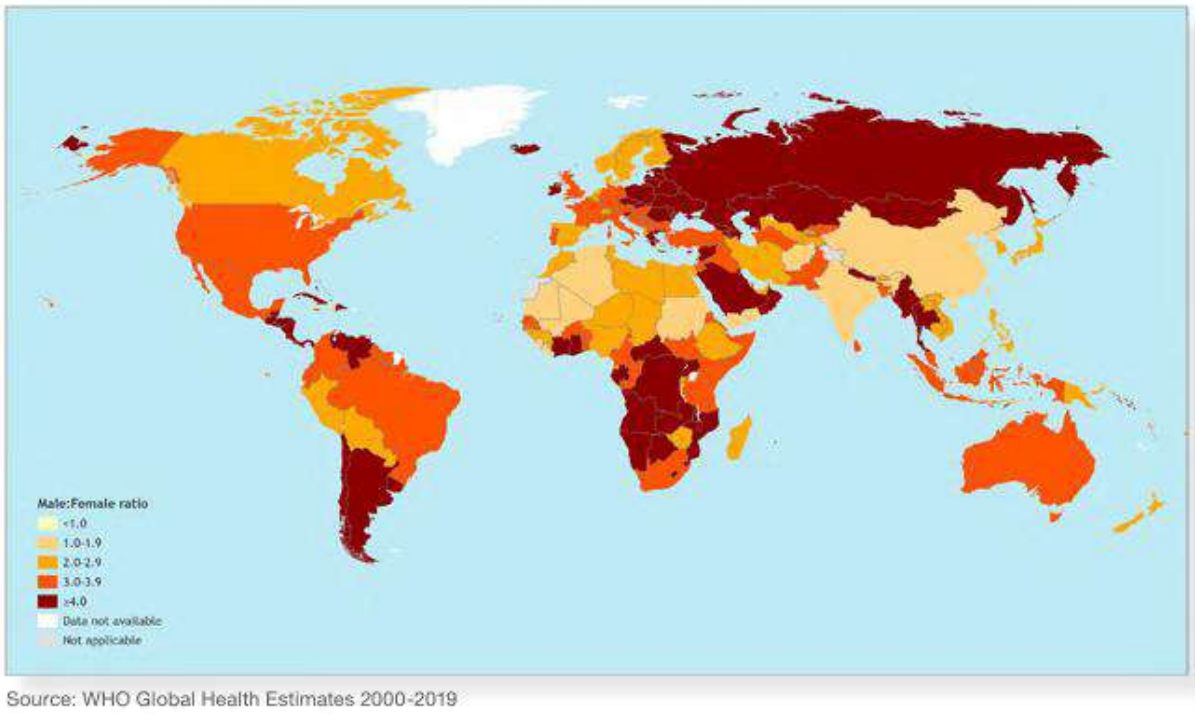


Figure 3: Male: female ratio of age standardised suicide rates 2019 (1)

Low income, unemployment, and poverty are conditions that can lead to self-harm or suicide. Conversely, suicides can result in a loss of productivity and income, further exacerbating a family's poverty(22).

Attitudes, values, and beliefs play a significant role in influencing an individual's decision to end their life(22,24). Due to the prohibition of suicide in Islam, reported suicide rates in Muslim contexts are low. In regions where Hinduism or secularized Christianity are prevalent, suicide rates hover around 10 per 100,000 population, such as in Italy (11.2) and India (9.6)(21).

Differences in methods of suicide are more pronounced between regions than between individual countries. The most prevalent methods are hanging and poisoning, with their prevalence varying significantly between countries, ranging from 8% to 70% for hanging and 8% to 83% for poisoning(22).

Substance-influenced suicides often occur after the intake of alcohol and/or sedative-hypnotic drugs, with 40-85% of these cases involving individuals who do not typically consume these substances(25). Cannabis does not constitute an independent risk factor for suicide. However, cocaine and methamphetamine are associated with suicide attempts in 20% of users and with suicidality in approximately two-thirds of users(25,26).

Half of all completed suicides are associated with depressive and other mood disorders. Compared to healthy individuals, those with these disorders have a 20-fold increased risk of suicide(27,28). Suicides associated with depression are more prevalent among the elderly, many of whom experience psychotic symptoms(29,30). In contrast, suicide attempts and suicides in individuals with bipolar disorder typically coincide with their first depressive episode, which generally occurs around the age of 25 years(31). Each comorbidity increases the risk of suicide across all mental disorders, with the highest risk observed in combinations involving psychotic disorders(22). Any chronic disease can be associated with an elevated risk of suicide. The literature specifically mentions conditions such as multiple sclerosis, epilepsy, systemic lupus erythematosus, asthma, and hemodialysis for kidney failure(22).

THYROID BIOMARKERS

Thyroid hormones and Suicide

Sinai et al (2009), in an observational, cross-sectional study of 100 euthyroid individuals who had attempted suicide, results suggested that in male suicide attempters, a lower T3/T4 ratio is associated with higher aggression and lower detachment personality traits(32).

Pompili et al (2012), in a prospective observational study of 439 cases assessed in the Emergency Department of an Italian Hospital, found that suicide attempters were less likely to have higher FT3 values compared to non-attempters(33).

Gokalp et al (2020), in a case-control study of 101 suicide attempters of the adolescent age group and 121 controls, obtained serum TSH levels were significantly lower in adolescents with a history of suicide attempts compared to controls and concluded that adolescents with subclinical hyperthyroidism had more suicide attempts than those in the control group(11).

In a retrospective cross-sectional study, S. V. Pisk et al (2023). examined adult psychiatric patients, including those with depressive, psychotic, and bipolar disorders, who had attempted suicide and those who had not. The study found lower total T3 levels in patients with depression and those who had attempted suicide, suggesting a link between thyroid hormones and serotonin regulation. This highlights the importance of routine thyroid function assessments to detect potential suicidality(34).

M. Bauer et al. (2002), in a review of the relationship between thyroid hormones and the brain serotonin (5-HT) system, noted that decreased thyroid hormones are associated with reduced serotonin responsiveness in humans. The effects of thyroid hormone administration in animals

with experimentally induced hypothyroid states include increased cortical 5-HT concentrations and disinhibition of cortical and hippocampal 5-HT release. In conclusion, the thyroid economy has a modulating impact on the brain's serotonin system(35).

Thyroid hormones changes after death

In a 1980 study, E. Rachut et al. investigated postmortem changes in serum levels of thyroxine, triiodothyronine, and parathormone. They found that these hormone levels showed irregular and variable patterns of decline, stability, or elevation after death. Thyroxine levels generally decreased, but this decline was statistically significant in only about half of the cases. Triiodothyronine levels exhibited more erratic behaviour, possibly due to conversion from thyroxine. Additionally, hormone levels from the inferior vena cava were often higher than those from the femoral veins, potentially due to diffusion from decomposing glands in the neck(36).

In a 1987 retrospective observational study, Wellby et al. examined serum samples taken before and after death in 36 cases of sudden infant death syndrome (SIDS). They found that serum total T3, free T3, and reverse T3 (rT3) levels significantly increased after death, while total T4, free thyroxine index (FTI), and free T4 levels significantly decreased. The authors concluded that the previously reported increases in T3 levels in SIDS cases are likely due to postmortem changes rather than a true biological effect(37).

In a 2020 cross-sectional observational study, N. Tani et al. analyzed blood samples from the left and right cardiac chambers and iliac veins, measuring serum levels of triiodothyronine (T3), thyroxine (T4), thyroglobulin (Tg), and thyroid-stimulating hormone (TSH). They found that serum T3 and T4 levels were elevated in cases of mechanical asphyxia and acute/subacute blunt head injury, indicating hypoxic and ischemic brain conditions compared to other causes of death. The study concluded that systemic advanced hypoxia/ischemia might trigger a rapid, TSH-independent release of T3 and T4 thyroid hormones in autopsy cases. These findings suggest that increased levels of thyroid-related hormones (T3 and T4) in the pathophysiological context could indicate systemic hypoxia/ischemia. ELISA method was used in this study(38).

Medicolegal importance of thyroid hormones changes

T. Ishikawa et al (2009) examined and compared postmortem serum and cerebrospinal fluid (CSF) levels of thyroid-stimulating hormone (TSH) in 120 medicolegal cases, including deaths from blunt injury, sharp instrument injury, fire fatalities, mechanical asphyxiation, drowning, poisoning, hypothermia, and acute ischemic heart disease. The study found that TSH levels in

both serum and CSF were significantly lower in cases of fatal hypothermia compared to other causes of death. Additionally, TSH immunopositivity in the adenohypophysis was lower in hypothermia cases, suggesting that the decrease in TSH levels is related to hypothalamic-adenohypophyseal dysfunction in hypothermia(39).

In a 1990 prospective observational study, Powner DJ et al. monitored serum levels of free thyroxine (fT4), thyroid-stimulating hormone (TSH), reverse T3 (rT3), cortisol, insulin, and lactate in 16 patients before and after brain death, and in 14 additional patients who were similarly compromised but did not become brain dead. They found that low fT3, normal fT4, and normal or high rT3 levels in most patients were consistent with a variant of the euthyroid sick syndrome, although TSH was elevated in some patients. This single finding does not justify diagnosing a hypothyroid state in these patients or administering thyroid hormone to brain-dead organ donors(40).

Muller et al. (1991) examined the levels of thyroglobulin, thyroxine, and triiodothyronine in heart blood samples taken during autopsies. They found that these hormone levels varied based on the cause of death. When compared to healthy living individuals, the postmortem levels of Tg, TT4, and TT3 showed significant differences. Specifically, Tg levels were higher in accident victims who survived for several days after their injuries. Thyroxine levels were elevated in cases of hanging, and TT3 levels were increased in both hanging and sudden natural deaths(41).

Postmortem biochemical investigations play a crucial role in forensic pathology, particularly in cases of mechanical asphyxia and neck compression. While postmortem biochemistry offers valuable insights, interpretation remains challenging due to postmortem changes and the lack of established reference ranges(42,43). Biochemical markers such as natriuretic peptides, creatine kinase, and procalcitonin can aid in determining the duration of traumatic processes(44).

Changes in thyroid in case of neck compression

Senol et al. (2008) conducted a cross-sectional observational study that included postmortem examinations of thyroid tissue and serum levels of various hormones, including triiodothyronine (fT3), thyroxine (fT4), thyrotropin (TSH), thyroglobulin, parathormone (PTH), calcitonin, and amylase. The study involved 24 cases of death by hanging and 25 cases of sudden death. Blood samples were taken by mixing blood from the right and left chambers of the heart. The normal ranges for these hormones were defined as 2.3–4.2 pg/mL for fT3, 0.89–1.80 ng/dL for fT4, and

0.35–5.5 IU/mL for TSH, with thyroglobulin levels considered normal between 1.4–78 ng/mL. The study found that the mean levels of free T3 (fT3) and thyroglobulin were significantly higher in cases of death by hanging compared to sudden death cases. This suggests that elevated postmortem levels of fT3 and thyroglobulin could indicate a vital reaction, meaning the person was alive during the hanging. The pressure applied to the neck during hanging is believed to cause the leakage of thyroglobulin and fT3 from the thyroid gland, leading to the elevated levels observed in the postmortem samples(8).

Palmiere et al. (2018) conducted a cross-sectional observational study investigating the postmortem stability of thyroglobulin, iodothyronines, calcitonin, and parathyroid hormone in blood. They compared these molecules in antemortem and postmortem serum samples from suicidal hanging cases and various other sites. The study found that postmortem levels of thyroglobulin, calcitonin, and parathyroid hormone remained stable, while total and free T4 levels decreased and total and free T3 levels increased. Mechanical force applied to the neck, as seen in hanging cases, may lead to elevated thyroglobulin levels in peripheral blood, although this is not always observed. Cases of hanging, manual, and ligature strangulation can show increased levels of thyroglobulin, total T3, and free T3 in postmortem blood samples, even without visible damage to the thyroid gland(45).

Marin Takaso et al. (2018) conducted a cross-sectional observational study to explore the relationship between blood thyroglobulin (Tg) levels and neck compression. They measured Tg levels in right cardiac blood using a chemiluminescence immunoassay in 256 autopsy cases. Among the 11 confirmed neck compression cases, the mean Tg level was 3155 ng/mL (range: 179-16,500 ng/mL). In the other cases, the mean Tg level was 4160 ng/mL (range: 0.3-139,000 ng/mL). There was no significant difference between the mean Tg levels of the two groups. However, the frequency of neck compression was significantly higher ($P < 0.05$) in the group with Tg levels ≥200 ng/mL. Tg levels in the right heart blood were about 10 times higher than those in the left heart blood and peripheral blood. Since high Tg levels were also found in half of the cases without neck compression, diagnoses should be made carefully based on autopsy findings and other information(46).

In a 1990 study by Keiji Tamak et al., researchers compared thyroglobulin (Tg) levels in cadavers with and without neck compression. They found that plasma Tg levels in all 36 cadavers without neck compression or injuries were below 200 ng/ml (averaging 73.6 ± 51.9 ng/ml), which is slightly higher than the levels found in living individuals (16.7 ± 11.8 ng/ml). Conversely, in 42

victims of asphyxia due to neck compression, most had plasma Tg levels exceeding 200 ng/ml (averaging 2190 ± 4300 ng/ml), with the highest recorded level being 24,600 ng/ml. The study suggested that Tg is released due to mechanical force on the thyroid gland during the agonal stage. Since thyroid diseases causing elevated Tg levels are rare in medicolegal cases, they should be ruled out through routine histological examination of the thyroid during autopsy(47).

In a 1984 laboratory-based experimental study, Y. Katsumata evaluated the use of bloodstains to diagnose mechanical asphyxia. Small pieces of bloodstains with known concentrations of thyroglobulin were extracted using 0.1 mL of distilled water. These extracts were then tested against antihuman thyroglobulin serum through precipitation-electrophoresis. Bloodstains with more than 1 μg/mL of thyroglobulin formed distinct precipitin lines for up to one month, while those with over 5 μg/mL maintained distinct lines for up to three months. The study was done on assumption that compression forces applied to the neck release thyroglobulin into circulation and it can be detected even in blood stains(48).

HISTOPATHOLOGY IN HANGING

The value of routine histological examination in hanging deaths is debated within forensic pathology. Some studies argue that histology offers limited insight into the cause, mechanism, and manner of death in straightforward hanging cases(49–51). Others contend that histological examination is crucial for assessing vitality and distinguishing between antemortem and postmortem hanging(52,53). Systematic histological examination has been found beneficial in a significant proportion of forensic autopsies, affecting cause and manner of death determinations and providing additional information about medical conditions(54). The debate also covers the need for internal examinations in hanging deaths. Some experts think external exams are enough for straightforward cases(50). However, others stress the importance of comprehensive internal and external exams, including histology, to detect and document injuries critical for determining the manner of death(55,56).

In a 2011 study, Magdalena Okłota and colleagues examined traumatic changes in the thyroid in vertical hanging cases with both open and closed nooses. They found that such changes could help confirm intravital hanging and aid in postmortem diagnoses when other signs of intravitality are not evident. Petechiae were observed in 33% of cases, appearing in 20% of closed noose hangings and 13% of open noose hangings. The study revealed no difference between open or closed nooses in terms of thyroid changes, nor any correlation between body position and thyroid injury. All cases showed thyroid hyperemia and segmental blood vessel expansion.

Histopathological analysis indicated that in one-third of cases with negative macroscopic findings, microscopic changes suggested the person was alive during hanging(57).

In 2022, Pathak Harish et al observed the submandibular, parotid, and thyroid glands in 41 hanging deaths. Histopathological examinations revealed congestion in 36 cases (87.8%) and focal interstitial infiltration in five cases (12.2%), mostly in partial hangings. They concluded that when other evidence is lacking, histopathological examination of the thyroid and salivary glands can be crucial for proving ante-mortem hanging. Longer hanging duration and body weight increase the likelihood of focal interstitial hemorrhages in these glands(58).

The study by Ikeda T in 2017 examined histopathological changes in the thyroid gland caused by systemic ischemia/hypoxia due to acute mechanical asphyxia or blunt head injury. Researchers compared these changes with other types of deaths, like drowning and sudden cardiac death, across 96 forensic autopsies. Findings highlighted that thyroid follicular destruction was more common in deaths from asphyxia and acute head injuries than other causes, and there was no relationship between blood thyroid hormone levels and follicular destruction. These changes point to systemic ischemia/hypoxia rather than mechanical neck injury (59).

In 1969, Yoshiya Okabe conducted a study where rabbits were subcutaneously injected with parathion, an organic phosphate chemical, twice weekly at doses of 4 mg, 8 mg, 16 mg, and 24 mg/kg. The histological changes in the thyroid were observed over 204 days across acute, subacute, and chronic stages:

- Acute stage: Mild vacuolar degeneration of follicular epithelia and dark intraluminal content were noted in rabbits given 24 mg/kg. Also observed were decreased staining grades, segmentation of arginophilic fibers, edematous swelling of stromal fibres, and interstitial expansion.
- Subacute stage: Slight irregularity in follicle size, atrophy, and vacuolar degeneration of epithelia occurred in those receiving 4-8 mg/kg. Changes in stromal and basement membrane fibers mirrored those in the acute stage.
- Chronic stage: Pronounced irregular follicle size, atrophy, and destruction were evident in the 24 mg/kg group. Desquamated epithelia and red blood cells were present in the lumen, with more prominent regressive changes(60).

CHAPTER 3: AIMS AND OBJECTIVES

AIMS AND OBJECTIVES

AIM:

- To study the autopsy, histopathology findings and biochemical markers such as thyroglobulin(Tg), FT3, FT4, Total T4 and TSH in cases of death due to hanging.

OBJECTIVES:

- To study histopathological changes in thyroid in death due to hanging in relation to other causes.
- To study changes of serum levels of thyroglobulin(Tg), FT4, FT3,TSH and Total T4 in deaths due to hanging in relation to other causes.
- To correlate histopathological and biochemical findings in deaths due to hanging.

CHAPTER 4: MATERIALS AND METHODS

MATERIALS AND METHODS

STUDY SETTING

The study was conducted in the Forensic Medicine and Toxicology Department, Pathology and Lab Medicine Department, and the Biochemistry Department at AIIMS Bhopal, a key institution in Madhya Pradesh, Central India.

STUDY DESIGN

Autopsy based observational study.

ETHICAL CLEARANCE

The study done after approval from the Institutional Human Ethics Committee for Student Research (IHEC-SR) of AIIMS Bhopal vide LOP letter no AIIMS/BPL/IHESR/July/22/PG/04 dated Feb 28, 2023.

STUDY POPULATION

Eligible deceased bodies brought to the mortuary of Department of Forensic medicine and Toxicology at AIIMS, Bhopal for medicolegal autopsy which fulfil the inclusion and exclusion criteria will be included in this study. The written informed consent will be taken from relatives or next to kin for the collection of study specimens.

PARTICIPANT'S SELECTION CRITERIA

Inclusion criteria:

- Medicolegal with age more than 10 years.
- Cases of deaths due to hanging confirmed by autopsy.
- Time since death within 24 hrs.

Exclusion criteria:

- Mutilated and decomposed bodies.
- Cases with unknown identity.
- Deaths due to neck compression other than hanging.
- Cases with established or known pathological disease of thyroid gland.
- Cases with evident gross pathology in thyroid gland.
- Cases where blood sample could not be obtained.

SAMPLE SIZE

During the study period from February 2022 to October 2022, all eligible deceased bodies arriving at the Mortuary of AIIMS, Bhopal for autopsy, meeting the inclusion and exclusion criteria, were included. This study covered 76 cases, with 38 deaths due to hanging and 38 control cases selected to match age and sex as closely as possible.

MATERIALS:

- Autopsy instruments.(Figure 4)
- 10 ml syringe.
- Centrifugation machine.(Figure 5)
- 1.5 ml tubes. (Figure 6)
- Compatible freezer (-80°C).
- Electronic balance machine.
- Micropipettes.
- Abbott Architect i2000 Chemistry Analyzer
- Sample container.
- 10% Normal buffered formalin.
- Automated tissue processor.
- Paraffin wax.
- Tissue staining solutions.
- Glass slides and cover slips.

- Light microscope.
- Fine test Human thyroglobulin ELISA Kit (Figure 7)
- 37°C incubator
- Microplate reader (Figure 8)

Figure 4: Autopsy instruments

Figure 5: Centrifugation machine

Figure 6: Preserved samples in 1.5 ml tubes

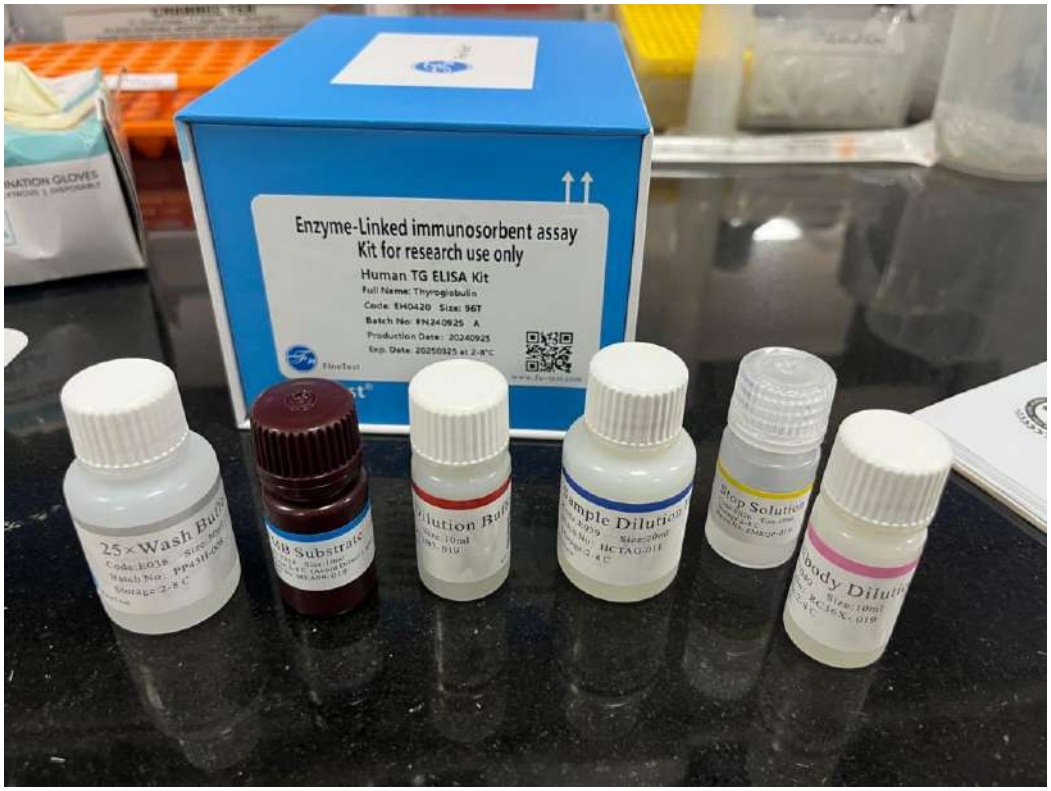


Figure 7: Human thyroglobulin ELIZA Kit

Figure 8: Microplate reader OD-450nm

PROCEDURE

Once the deceased body met the inclusion and exclusion criteria, the next of kin and family members were informed about the study and provided with a printed patient information sheet (Annexure I). Blood and thyroid gland tissue were collected only after obtaining a written consent form (Annexure II) duly filled and signed by the next of kin. Cases were initially selected based on the history provided by relatives or police before starting the autopsy.

External Examination:

The body underwent a thorough external examination to identify any abnormalities or injuries.

In cases of suspected suicide by hanging, a detailed inspection of the neck region was conducted to document the presence and characteristics of any ligature marks.

If a ligature was found in situ (still in place), it was carefully cut away from the knot and examined.

Opening the Body:

The internal examination began with the opening of the thoracic and cranial cavities.

Standard dissection procedures were followed to ensure a systematic approach.

Organ Examination:

Before the organs were removed, they were weighed and re-examined for any gross abnormalities.

Blood Collection

Blood samples were retrieved using 10ml syringes and transferred to yellow blood collection vials designed for serum separation.

Blood sampling sites: Blood from peripheral blood vessels for thyroglobulin to eliminate postmortem diffusion. Cardiac blood for all the other of the assays.

The whole blood sample was left at room temperature for 2 hours. After centrifuging for 20 minutes at 3000rpm, the serum was collected for immediate detection whenever possible. Alternatively, the serum was aliquoted and stored at -20°C or -80°C for future use.

Dissection of Organs and neck dissection:

Each organ was removed and dissected according to standard protocols.

After all organs had been removed, the neck was opened in a bloodless field, dissecting layer by layer to ensure a detailed examination. (Figure 9)

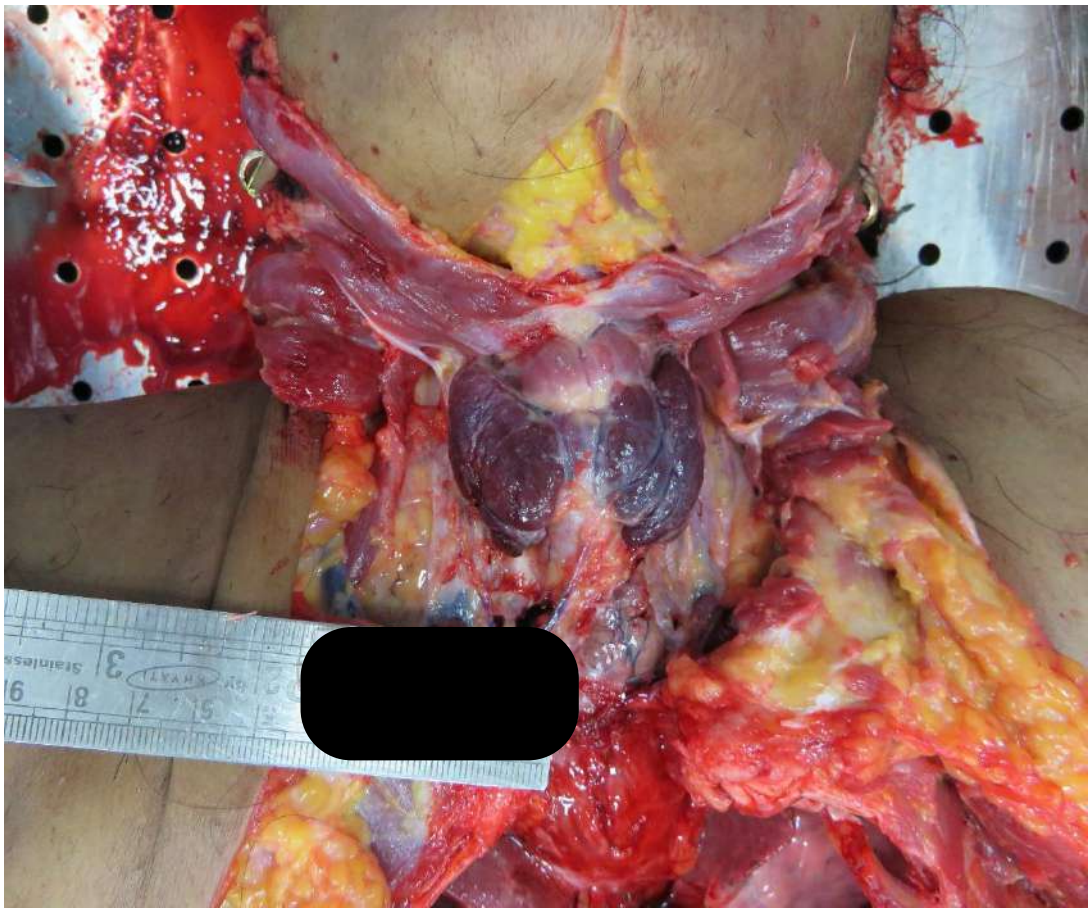


Figure 9: Neck dissection in bloodless field showing congested thyroid gland

Dissection of thyroid gland:

The thyroid gland was then inspected, its gross appearance documented (Figure 10), and carefully excised using fine dissection techniques. Its weight was measured with a digital balance. The thyroid gland was obtained, and at least half of the lobe nearest to the ligature mark was placed in large plastic containers. This thyroid sample was fixed in 10% neutral buffered formalin.

Figure 10: Gross examination of thyroid gland

The specimens were meticulously collected to ensure that the body's appearance was maintained post-autopsy reconstruction. All samples and materials were discarded following the institute Biomedical waste disposal guidelines.

TISSUE PROCESSING(61)**:**

Dehydration

Graded alcohol is utilized in dehydration processes to eliminate free water while preserving bound water.

Clearing and Infiltration

Xylene is used to remove the alcohol content, facilitating the subsequent paraffin process to ensure thorough infiltration. After clearing, tissue sections are infiltrated (impregnated) with paraffin wax to support the tissue for thin sectioning. Once infiltration is complete, tissues are embedded into a paraffin wax block to enable sectioning on a microtome.

Microtomy

The paraffin-embedded tissue block was sectioned using microtomy into slices 4-5 micrometres thick. The tissue sections were then floated on a warm water bath to remove any wrinkles and to aid in placing the tissue onto the glass slide.

HISTOPATHOLOGICAL EXAMINATION (HAEMATOXYLIN AND EOSIN STAINING PROCEDURE)(61)**:**

Staining Method:

The histological staining method used to observe the cell and tissue structure of the samples was haematoxylin and eosin (H&E) staining.

Deparaffinization:

The slides were initially deparaffinized by briefly passing them over a flame and then immersing them in xylene. This process was repeated until all the paraffin wax was successfully removed from the tissue sections.

Dehydration:

After the xylene was drained, the tissue slices were hydrated by passing them through a series of alcohol baths with decreasing concentrations (100%, 90%, 80%, 70%) followed by water.

Nuclear Staining:

The tissue sections were stained with haematoxylin for 3-5 minutes to highlight the nuclei.

Washing:

The slides were rinsed under running water until the sections appeared blue, which typically took no more than 5 minutes.

Differentiation:

Excess haematoxylin dye was removed by briefly immersing the slides in 1% acid alcohol (1% HCl in 70% alcohol).

Counterstaining:

The slides were counterstained with 1% Eosin Y for 10 minutes to provide contrast to the haematoxylin-stained nuclei.

Rinsing:

The slides were rinsed with tap water for 1 to 5 minutes to remove excess eosin.

Dehydration:

The slides were dehydrated by passing them through alcohol baths of increasing concentration.

Clearing:

The slides were cleared by immersing them in two xylene baths.

Mounting:

Finally, the specimens were mounted using DPX or another suitable mounting medium.

Microscopic Examination:

Following these steps, the specimens were examined under a compound microscope for optimal results. The histopathological examination of the slides was carried out in the Pathology Lab.

ELISA FOR THYROGLOBULIN ESTIMATION

Technique: Sandwich ELISA (Figure 11)

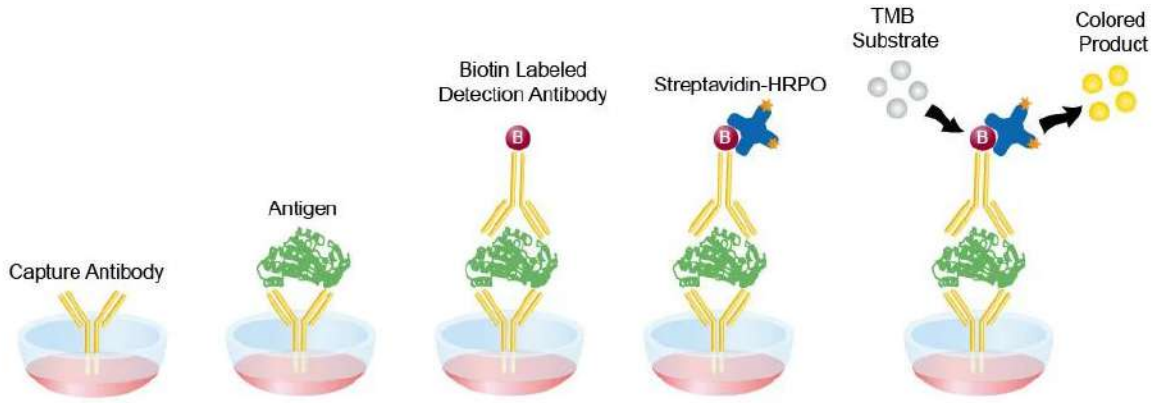


Figure 11: Pictorial representation of Sandwich ELISA (62)

Product specification: FineTest® Human TG(Thyroglobulin) ELISA Kit

Range: 0.78-50ng/ml

Sensitivity: 0.469ng/ml

DETAILED ELISA ASSAY PROCEDURE(38,63,64)

1. **Setup**
    - Arranged and labelled standard, samples, and wells on the pre-coated plate.
    - Measured each standard and sample.
2. **Performed 10-Fold Dilution**
    - Added 12 µL of the sample into 108 µL of sample diluent and mixed gently.
3. **Loaded Standards and Samples**
    - Dispensed 100 µL of zero tube, 1st tube, 2nd tube, 3rd tube, and 4th tube into each standard well. Zero tube contains a concentration of 50ng/mL.
    - Added 100 µL of sample dilution buffer to the control well.
    - Added 100 µL of samples into each sample well.
    - Sealed the plate and incubated for 90 minutes at 37°C.

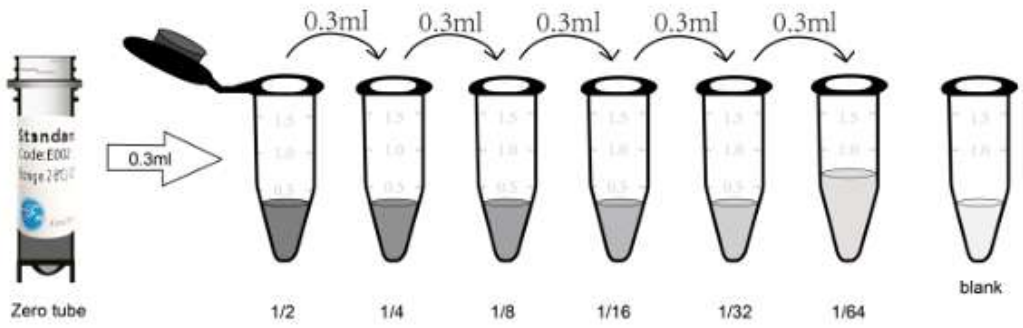


Figure 12: Standard preparation.(64)

4. **Washed Twice**
    - Removed cover and absorbed liquid.
    - Added 350 µL of wash buffer to each well.
    - Discarded liquid and repeated washing step twice.
5. **Added Biotin-labeled Antibody**
    - Added 100 µL of biotin-labeled antibody working solution to each well.

- Sealed the plate and incubated for 60 minutes at 37°C.

6. **Washed Three Times**
    - Removed cover and absorbed liquid.
    - Added 350 µL of wash buffer to each well and immersed for 1 minute.
    - Discarded liquid and repeated washing step three times.
7. **Added HRP-Streptavidin Conjugate (SABC)**
    - Added 100 µL of SABC working solution to each well.
    - Sealed the plate and incubated for 30 minutes at 37°C.
8. **Washed Five Times**
    - Removed cover and washed the plate with wash buffer five times.
9. **Added TMB Substrate**
    - Added 90 µL of TMB Substrate to each well.
    - Sealed the plate and incubated at 37°C in the dark for 10-20 minutes.(Figure 13)
    - Preheated the microplate reader for 15 minutes.

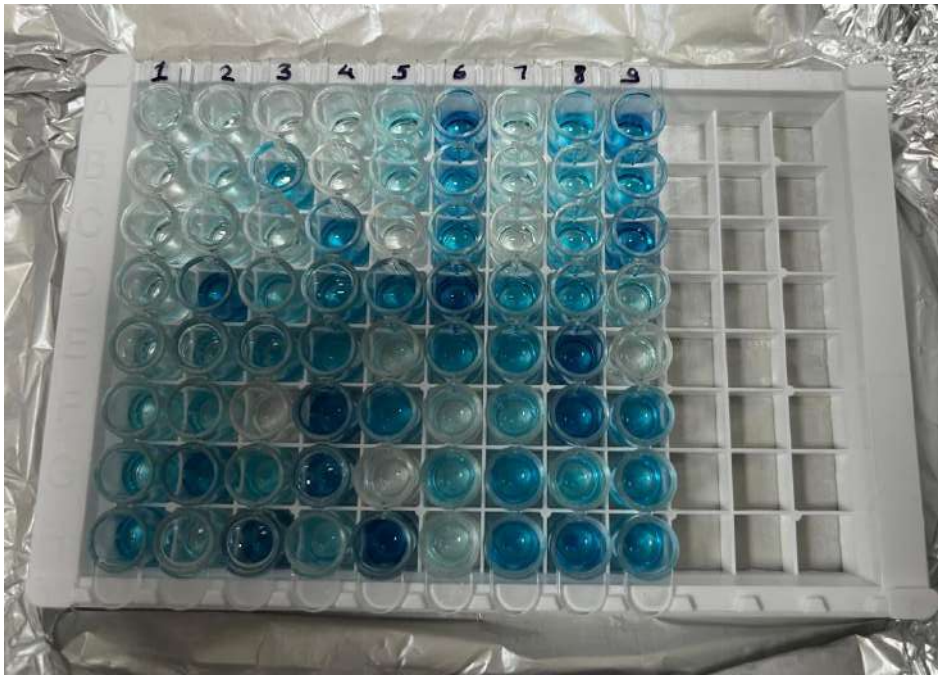


Figure 13: Microplate showing color reaction after adding TMB Substrate.

10. **Stopped Reaction**

- Added 50 µL of stop solution to each well.
- The color turns yellow immediately. (Figure 14)

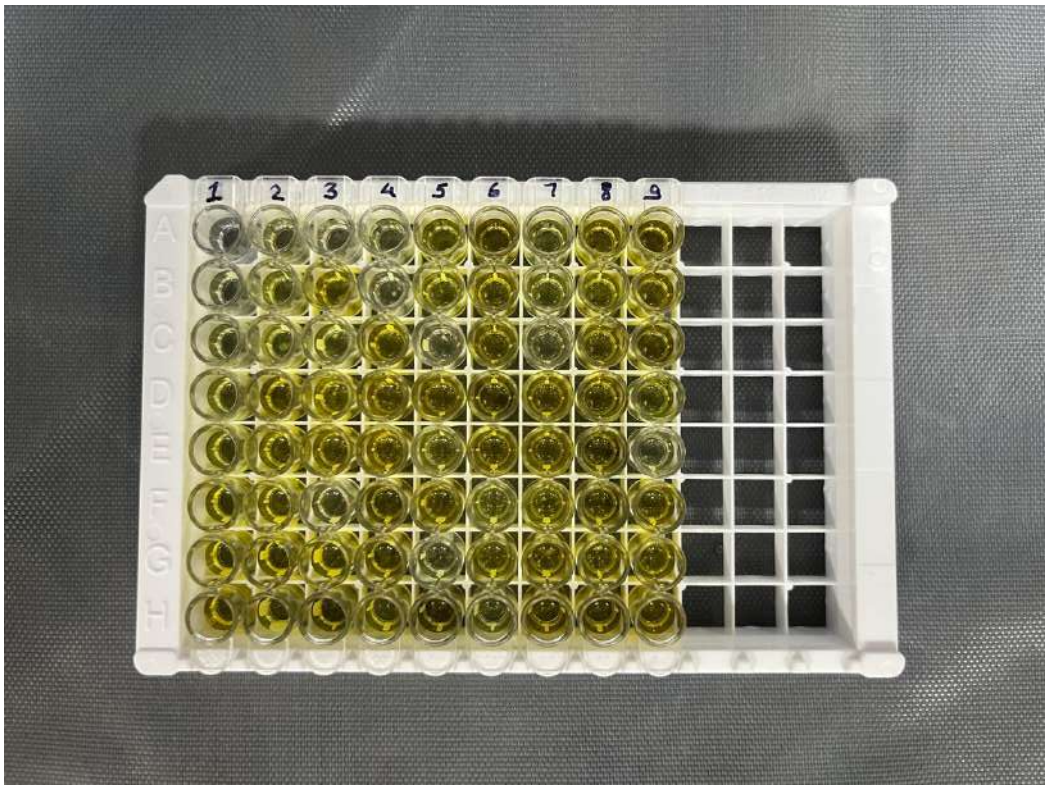


Figure 14: Final microplate used for reading

11. **OD Measurement**

- Read the O.D. absorbance at 450 nm using a microplate reader and calculated the results.

MEASUREMENT OF T3, TT4 AND FT4

We used the Abbott Architect i2000 Chemistry Analyzer, which employed chemiluminescence immunoassay (CLIA) technology(46), for our research. This advanced system allowed us to measure thyroid hormones such as T3, T4, FT4, and TSH with high precision and sensitivity.

Figure 15: Abbott Architect i2000 Chemistry Analyzer

Table 1: Normal ranges of thyroid biomarkers.(8,38)

FT4(ng/dl)	TSH(uIU/ml)	T3(ng/ml)	TT4(ng/dl)	Tg(ng/ml)
0.89- 1.8	0.35- 5.5	0.8- 1.6	6.1- 12.4	0 - 78

STATISTICAL ANALYSIS

- The data of each case was saved and stored in a Microsoft Excel sheet.
- Discriminate functional analysis was done by using R software for obtaining statistical parameters such as proportion, mean and standard deviation.
- P value of <0.05 considered as significant.

CHAPTER 4: OBSERVATION AND RESULTS

OBSERVATION AND RESULTS

During the study duration a total of 1002 autopsies were conducted at the mortuary of AIIMS Bhopal. Cases met the inclusion and exclusion criteria were included in the study. The sampling method was convenient sampling which were 71 (37 hanging and 39 non suicidal cases for control). Rest of cases were not fulfilling the inclusion criteria.

CAUSE OF DEATH OF TOTAL CASES

Table 2:Cause of death of entire study group

Characteristic	**N = 76**[1]
Cause of death	
Brain Pathology	1 (1.3%)
Burn Injury	3 (3.9%)
Cardiac Pathology	4 (5.3%)
Electrocution	1 (1.3%)
Hanging	37 (49%)
Head Injury	7 (9.2%)
Kept open	1 (1.3%)
Liver Pathology	1 (1.3%)
Lung pathology	2 (2.6%)
Multiple injuries	13 (17%)
Poisoning	5 (6.6%)
Snake bite	1 (1.3%)

[1] n (%)

Out of a total of 76 cases, hanging, accounted for 37 (49%) cases. Multiple injuries were the most common cause of death in controls, with 13 (17%) cases. Head injuries led to 7 (9.2%) deaths, while poisoning was responsible for 5 (6.6%) cases. Burns and cardiac pathology caused 3 (3.9%) and 4 (5.3%) deaths, respectively. There were also instances of death by electrocution (1.3%), brain pathology (1.3%), liver pathology (1.3%), lung pathology (2.6%), and snake bites (1.3%).

TIME SINCE DEATH OF TOTAL CASES

Table 3: Time since death of entire study group

Characteristic	**N = 76**[1]
Time since death (hrs)	
6	**31 (41%)**
12	**34 (45%)**
24	**11 (14%)**
[1] n (%)	

Out of a total of 76 cases, 31 (41%) bodies were brought for autopsy within 6 hours after death, 34 (45%) within 12 hours, and 11 (14%) within 24 hours.

Table 4: Time since death comparison between cases and controls

Characteristic	**Overall** N = 76[1]	**6 hr** N = 31[1]	**12 hr** N = 34[1]	**24 hr** N = 11[1]
Study group				
Case	37 (49%)	9 (29%)	18 (53%)	10 (91%)
Control	39 (51%)	22 (71%)	16 (47%)	1 (9.1%)
[1] n (%)				

In the 6-hour group, 9 were hanging cases and 22 were controls. In the 12-hour group, 18 were hanging cases and 16 were controls. For the 24-hour group, 10 were hanging cases and 1 was a control.

DEMOGRAPHY

AGE AND SEX DISTRIBUTION OF TOTAL CASES

Table 5: Age and sex distribution of entire study group

Characteristic	**N = 76**
Age	**30 (22, 38)**
Age group	
< 20	**16 (21%)**
> 40	**16 (21%)**
20-40	**44 (58%)**
Sex	
Female	**24 (32%)**
Male	**52 (68%)**
[1] n (%); Median (Q1, Q3)	

Out of a total of 76 cases, the age distribution showed that 16 (21%) individuals were below 20 years, 44 (58%) were between 20 and 40 years, and 16 (21%) were above 40 years. The median age was 30 years, with a range between 22 and 38 years. Regarding gender, 24 (32%) of the cases were female, while 52 (68%) were male. This data highlights the prominence of the 20-40 age group and the higher incidence among males.

In case of hanging:

Table 6: Age and sex distribution of hanging cases

Characteristic	**N = 37**[1]
Age	30 (10)
Age group	
< 20	8 (22%)
> 40	7 (19%)
20-40	22 (59%)
Sex	
Female	13 (35%)
Male	24 (65%)

Characteristic	N = 37[1]
[1] Mean (SD); n (%)	

Out of a total of 37 hanging cases, the mean age was 30 years with a standard deviation of 10 years. Age distribution showed that 8 (22%) individuals were below 20 years, 22 (59%) were between 20 and 40 years, and 7 (19%) were above 40 years. Regarding gender, 13 (35%) of the cases were female, while 24 (65%) were male. This data highlights the prominence of the 20-40 age group and the higher incidence among males.

EDUCATIONAL STATUS

Table 7: Educational status of entire study group

Characteristic	N = 76
Education	
Higher studies	**7 (9.2%)**
Highschool	**32 (42%)**
Primary or below	**23 (30%)**
Secondary	**14 (18%)**
[1] n (%); Median (Q1, Q3)	

Out of a total of 76 cases, 7 (9.2%) individuals had higher studies, 32 (42%) had completed high school, 23 (30%) had primary or lower education, and 14 (18%) had secondary education. This data shows a higher incidence of cases among those with high school education, followed by primary and secondary education.

In case of hanging:

Table 8: Educational status of hanging cases

Characteristic	N = 37[1]
Education	
Higher studies	3 (8.1%)
Higher secondary	9 (24%)
Highschool	14 (38%)

Characteristic	N = 37[1]
Primary or below	11 (30%)
[1] Mean (SD); n (%)	

Out of a total of 37 hanging cases, 14 (38%) individuals had attended high school, 11 (30%) had primary or lower education, 9 (24%) had higher secondary education, and 3 (8.1%) had pursued higher studies. This data highlights a higher incidence of cases among those with high school education.

RELIGION

Table 9: Religion of entire study group

Characteristic	N = 76
Religion	
Hindu	**72 (95%)**
Non-Hindu	**4 (5.3%)**
[1] n (%); Median (Q1, Q3)	

Out of a total of 76 cases, 72 (95%) individuals were Hindu, while 4 (5.3%) were non-Hindu. This indicates a predominant representation of Hindu individuals in the dataset.

In case of hanging:

Table 10: Religion of hanging cases

Characteristic	N = 37[1]
Religion	
Hindu	36 (97%)
Non-Hindu	1 (2.7%)
[1] Mean (SD); n (%)	

Out of a total of 37 hanging cases, 36 (97%) individuals were Hindu, while 1 (2.7%) was non-Hindu.

OCCUPATIONAL AND MARITAL STATUS

Table 11: Occupational status of entire study group

Characteristic	**N = 76**
Occupation	
Housewife	**9 (12%)**
Non-skilled	**40 (53%)**
Skilled	**8 (11%)**
Student	**19 (25%)**
Married	**48 (63%)**
[1] n (%); Median (Q1, Q3)	

Out of a total of 76 cases, 40 (53%) were non-skilled workers, 19 (25%) were students, 9 (12%) were housewives, and 8 (11%) were skilled workers. Regarding marital status, 48 (63%) of the individuals were married.

In case of hanging:

Table 12: Occupational status of hanging cases

Characteristic	**N = 37**[1]
Occupation	
Housewife	4 (11%)
Non-skilled	18 (49%)
Skilled	6 (16%)
Student	9 (24%)
Married	24 (65%)
[1] Mean (SD); n (%)	

Regarding occupation, 18 (49%) were non-skilled workers, 9 (24%) were students, 6 (16%) were skilled workers, and 4 (11%) were housewives. Regarding marital status, 24 (65%) of the individuals were married.

PERSONAL HISTORY

Table 13: Personal history of entire study group

Characteristic	**N = 76**[1]
Smoking status	18 (24%)
Alcohol status	27 (36%)
Chewable tobacco status	36 (47%)
Drugs status	3 (3.9%)
[1] n (%); Median (Q1, Q3)	

Out of a total of 76 cases, 36 (47%) individuals used chewable tobacco, 27 (36%) consumed alcohol, 18 (24%) were smokers, and 3 (3.9%) used drugs. This data shows a higher incidence of chewable tobacco and alcohol consumption.

In cases of hanging:

Table 14: Personal history of hanging cases

Characteristic	**N = 37**[1]
Smoking	9 (24%)
Alcohol	15 (41%)
Tobacco	15 (41%)
Drugs	3 (8.1%)
[1] Mean (SD); n (%)	

Out of a total of 37 hanging cases, 24 (65%) individuals were married. Regarding substance use, 15 (41%) consumed alcohol, 15 (41%) used tobacco, 9 (24%) were smokers, and 3 (8.1%) used drugs.

PAST HISTORY

Table 15: Past history of entire study group

Characteristic	**N = 76**[1]
Previous surgical treatment	3 (3.9%)
Hypertension status	7 (9.2%)
Diabetes status	1 (1.3%)
History of mental illness	3 (3.9%)
Previous medical treatment	4 (5.3%)
[1] n (%); Median (Q1, Q3)	

Out of a total of 76 cases, 7 (9.2%) individuals had hypertension, 4 (5.3%) had previous medical treatment, 3 (3.9%) had a history of mental illness, and 3 (3.9%) had undergone previous surgical treatment. Diabetes was present in 1 (1.3%) case.

In cases of hanging:

Table 16: Past history of hanging cases

Characteristic	**N = 37**[1]
Previous Surgical treatment	2 (5.4%)
Hypertension	3 (8.1%)
Diabetes	1 (2.7%)
History of mental illness	3 (8.1%)
Previous medical treatment	2 (5.4%)
[1] Mean (SD); n (%)	

In terms of previous medical history, 3 (8.1%) had hypertension, 2 (5.4%) had previous surgical treatment, 3 (8.1%) had a history of mental illness, and 2 (5.4%) had previous medical treatment. Diabetes was present in 1 (2.7%) case.

AUTOPSY FINDINGS

GENERAL FINDINGS

Table 17: general autopsy findings of entire study group

Characteristic	**Overall** N = 76[1]	**Case** N = 37[1]	**Control** N = 39[1]	**p-value**[2]
Facial congestion				<0.001
Absent	36 (47%)	2 (5.4%)	34 (87%)	
Present	40 (53%)	35 (95%)	5 (13%)	
Cyanosis				<0.001
Absent	34 (45%)	8 (22%)	26 (67%)	
Present	42 (55%)	29 (78%)	13 (33%)	
Petechiae				0.9
Absent	56 (74%)	27 (73%)	29 (74%)	
Present	20 (26%)	10 (27%)	10 (26%)	
[1] n (%)				
[2] Pearson's Chi-squared test				

Out of a total of 76 cases, 36 individuals (47%) showed no signs of facial congestion, whereas 40 individuals (53%) did. Within the group of hanging cases, 35 individuals (95%) presented facial congestion, as opposed to only 2 individuals (5.4%) in the control group. This difference is statistically significant, as indicated by a p-value of <0.001. Cyanosis was present in 42 individuals (55%) overall, with 29 cases (78%) in the hanging group and 13 cases (33%) in the control group, also showing a significant difference with a p-value of <0.001. Petechiae were present in 20 individuals (26%) overall, with 10 cases (27%) in both the hanging and control groups, indicating no significant difference, as reflected by a p-value of 0.9. Pearson's Chi-squared test was used for the statistical analysis.

AUTOPSY FINDINGS IN CASES OF HANGING:

MATERIAL USED

Table 18: Characteristic of ligature material

Characteristic	**N = 37**[1]
Ligature Material	
Cotton	11 (31%)
Jute	2 (5.7%)
Nylon	4 (11%)
Synthetic	18 (51%)
Unknown	2
Ligature Material Type	
Hard	11 (31%)
Soft	24 (69%)
Unknown	2

[1] n (%); Mean (SD)

Out of a total of 37 hanging cases, the most frequently used ligature material was synthetic, accounting for 18 (51%) cases. Cotton was used in 11 (31%) cases, nylon in 4 (11%) cases, and jute in 2 (5.7%) cases. Soft ligature material was used in 24 (69%) cases, while hard ligature material was used in 11 (31%) cases. The ligature material was unknown in 2 cases.

CHARACTERISTICS OF HANGING

Table 19: Characteristic of hanging

Characteristic	**N = 37**[1]
Type of hanging	
Complete	33 (89%)
Partial	4 (11%)

Characteristic	N = 37[1]
[1] n (%); Mean (SD)	

Regarding the type of hanging, 33 (89%) cases were complete hangings, while 4 (11%) were partial hangings.

Table 20: Characteristic of Ligature mark

Characteristic	N = 37[1]
Ligature mark impression	
Faint	3 (8.1%)
Grooved	11 (30%)
Prominent	23 (62%)
[1] n (%); Mean (SD)	

In terms of ligature mark impressions, 23 (62%) cases had prominent impressions, 11 (30%) had grooved impressions, and 3 (8.1%) had faint impressions.

Table 21: Knot position

Characteristic	N = 37[1]
Knot position	
Anterior	1 (2.7%)
Lateral	9 (24%)
Posterior	27 (73%)
[1] n (%); Mean (SD)	

Out of a total of 37 hanging cases, the knot position was posterior in 27 (73%) cases, lateral in 9 (24%) cases, and anterior in 1 (2.7%) case.

GROSS APPEARANCE OF THYROID GLAND

Table 22: Gross appearance of Thyroid

Characteristic	**N = 37**[1]
Thyroid gross appearance	
Congested	23 (62%)
Normal	14 (38%)
Thyroid weight	14.2 (3.3)
[1] n (%); Mean (SD)	

Out of a total of 37 hanging cases, the gross appearance of the thyroid was congested in 23 (62%) cases and normal in 14 (38%) cases. The mean thyroid weight was 14.2 grams with a standard deviation of 3.3 grams.

Table 23: Weight of Thyroid

Thyroid weight	**Mean (Range) in gms**
Male	15.58 **(**8-21)
Female	11.61 **(**8-16**)**

For females, the mean thyroid weight is 11.61 grams, with a range of 8 to 16 grams. For males, the mean thyroid weight is 15.58 grams, with a range of 8 to 21 grams. This data indicates a notable difference in thyroid weight between genders, with males generally having a higher thyroid weight.

HISTOPATHOLOGY OF THYROID IN CASES OF HANGING

Table 24: Histopathological findings of Thyroid

Histopathological findings	**N = 37**[1]
Follicular destruction	31 (84%)
Vacuolation in epithelial cells	32 (86%)
Crystallization in the follicle	3 (8.1%)
Engorgement of stromal capillaries.	34 (92%)
Parenchymatous hemorrhage	11 (30%)
Intrafollicular aggregates of RBC's	6 (16%)
Variation in follicular size	7 (19%)
Metachromasia	15 (41%)
Inflammation	13(34.7%)
Acute inflammation	1 (2.7%)
Chronic inflammation	12 (32%)
[1] n (%)	

Out of a total of 37 hanging cases, follicular destruction was present in 31 cases (84%). Vacuolation in epithelial cells was present in 32 cases (86%). Crystallization in the follicle was present in 3 cases (8.1%). Engorgement of stromal capillaries was present in 34 cases (92%). Parenchymatous hemorrhage was present in 11 cases (30%). Intrafollicular aggregates of RBCs were present in 6 cases (16%). Variation in follicular size was present in 7 cases (19%). Metachromasia was present in 15 cases (41%). Inflammation was present in 13 cases (35%), with acute inflammation in 1 case (2.7%) and chronic inflammation in 12 cases (32%). This analysis highlights significant patterns in the thyroid histopathology of hanging cases, particularly the high occurrence of engorgement of stromal capillaries and follicular destruction.

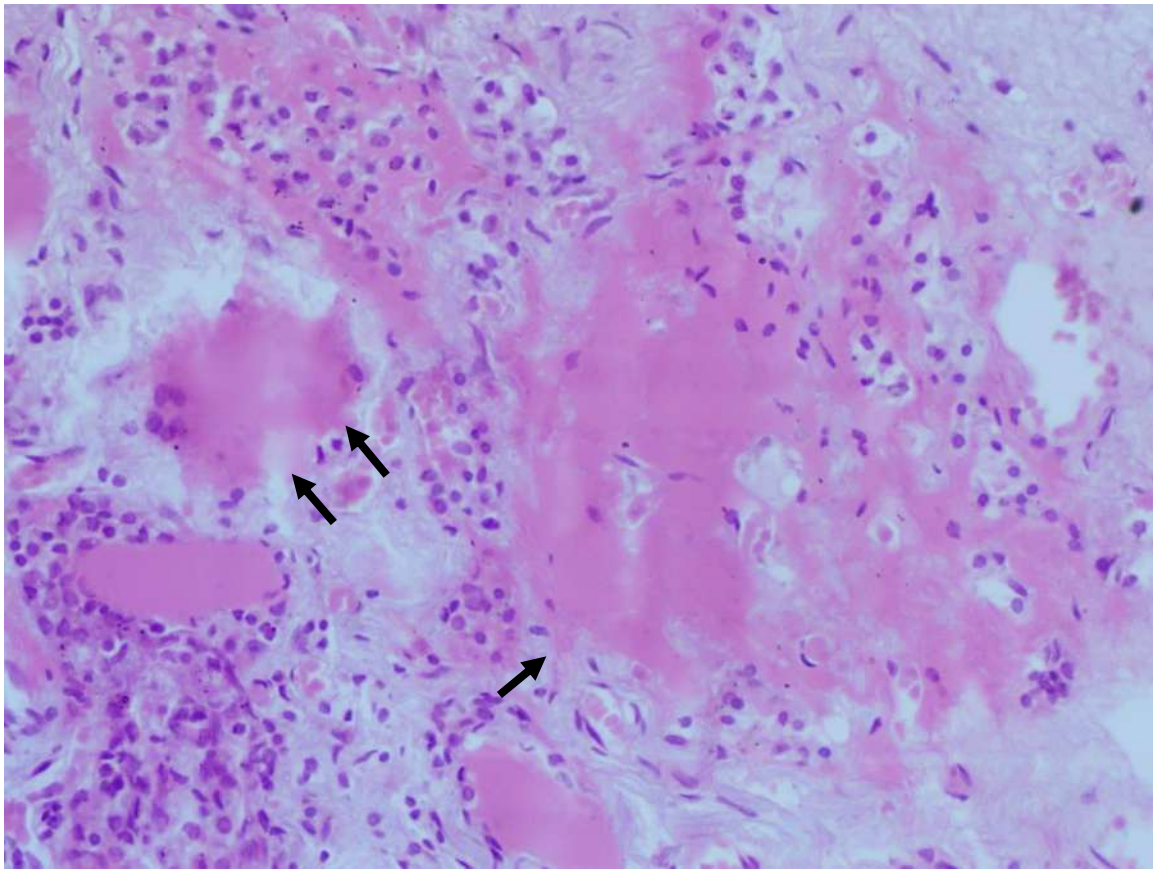


Figure 16 Microphotograph showing follicular destruction (Arrow) (H&E: 40x)

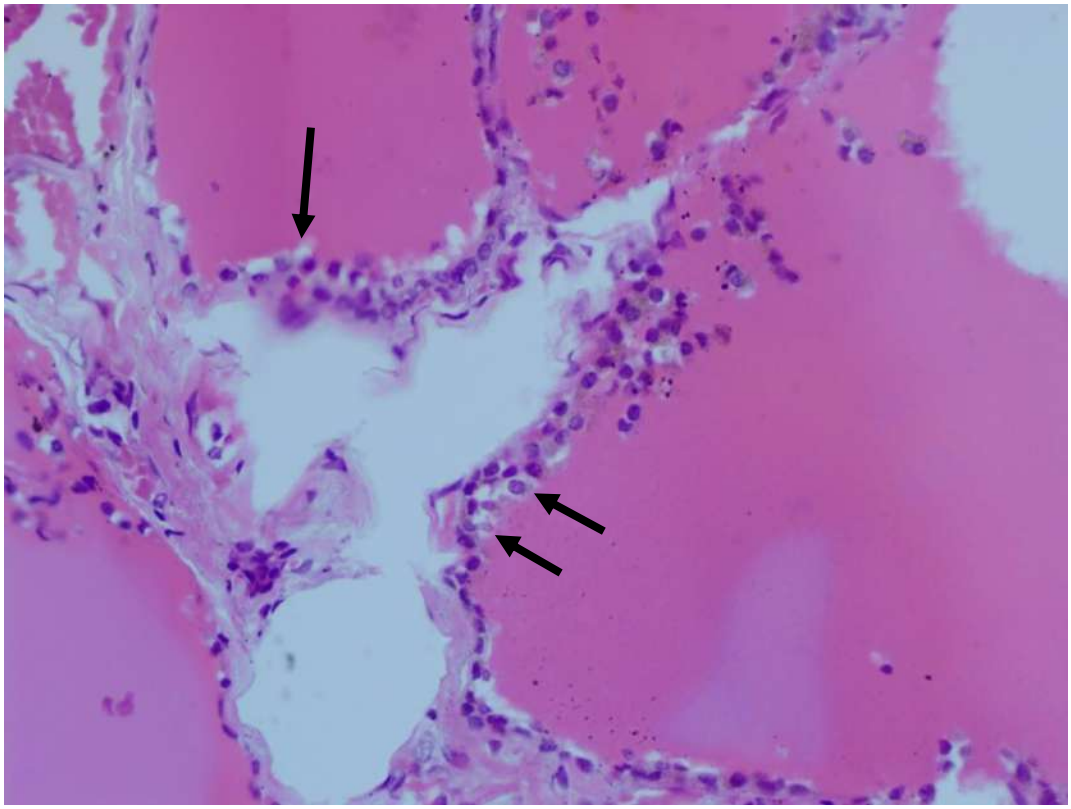


Figure 17 Microphotograph showing cytoplasmic vacuolation in epithelial cells (Arrows) (H&E: 40x)

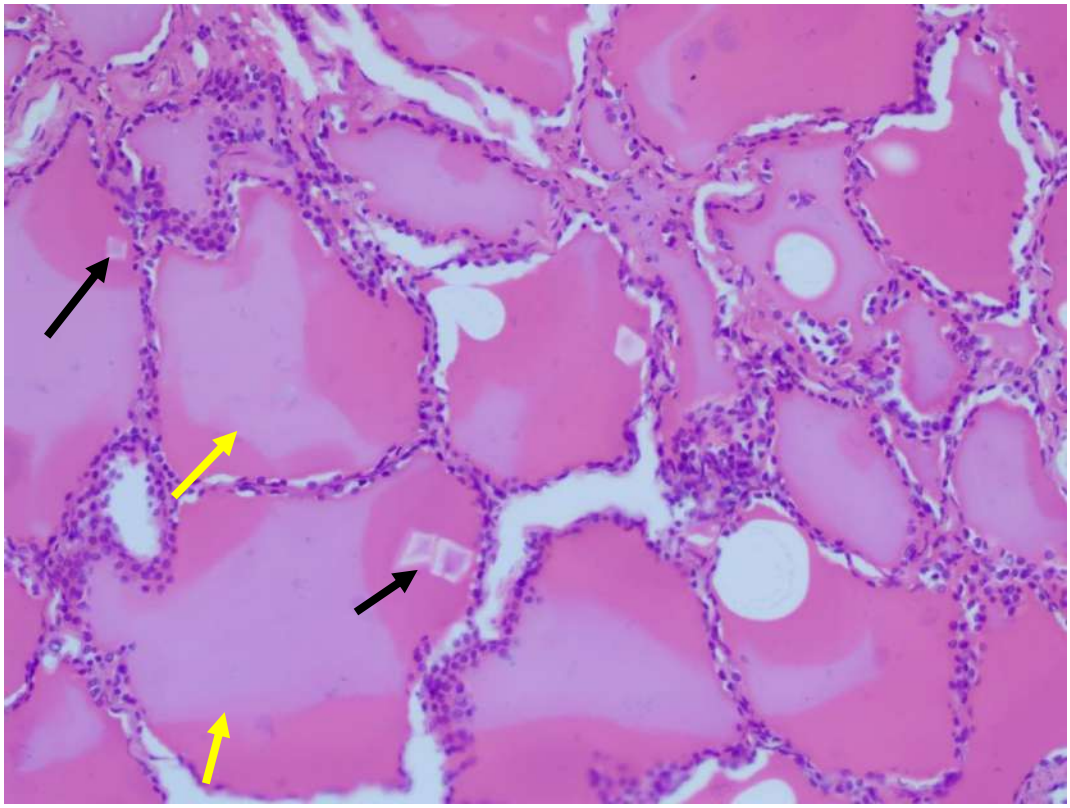


Figure 18 Microphotograph showing crystal formation (crystallisation) in the follicle (Black Arrow) & Metachromasia (Yellow Arrow). (H&E: 10x)

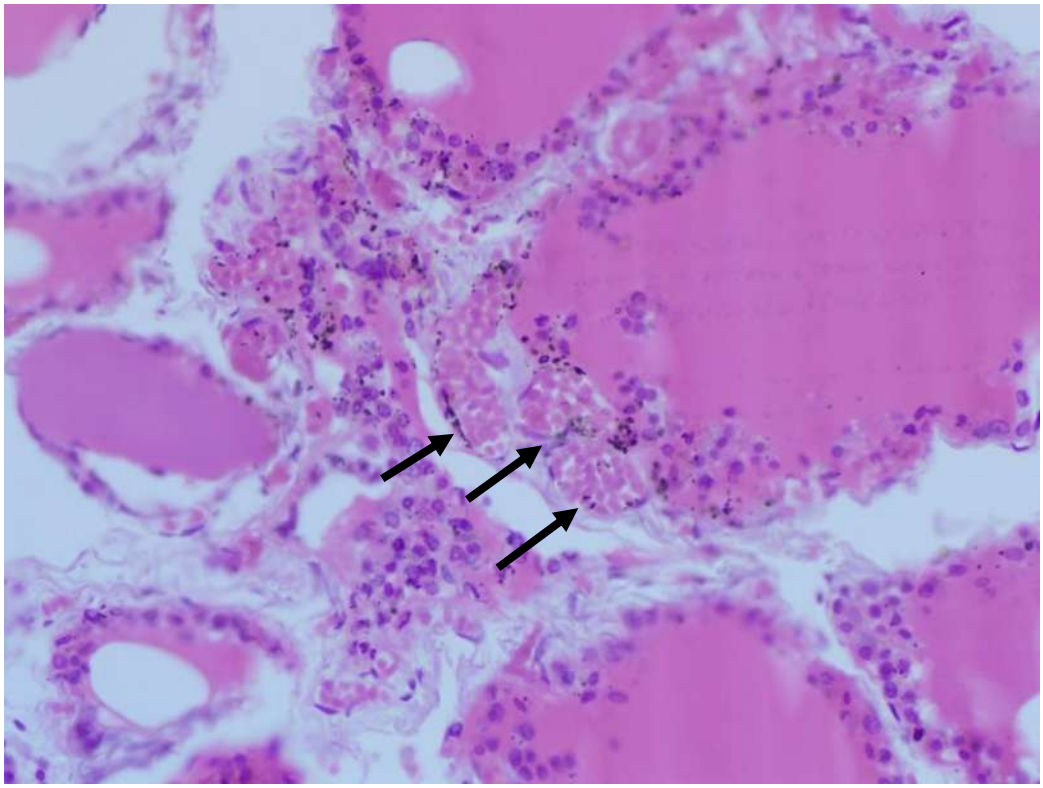


Figure 19 shows Engorgement of stromal capillaries (Arrows) (H&E 40x).

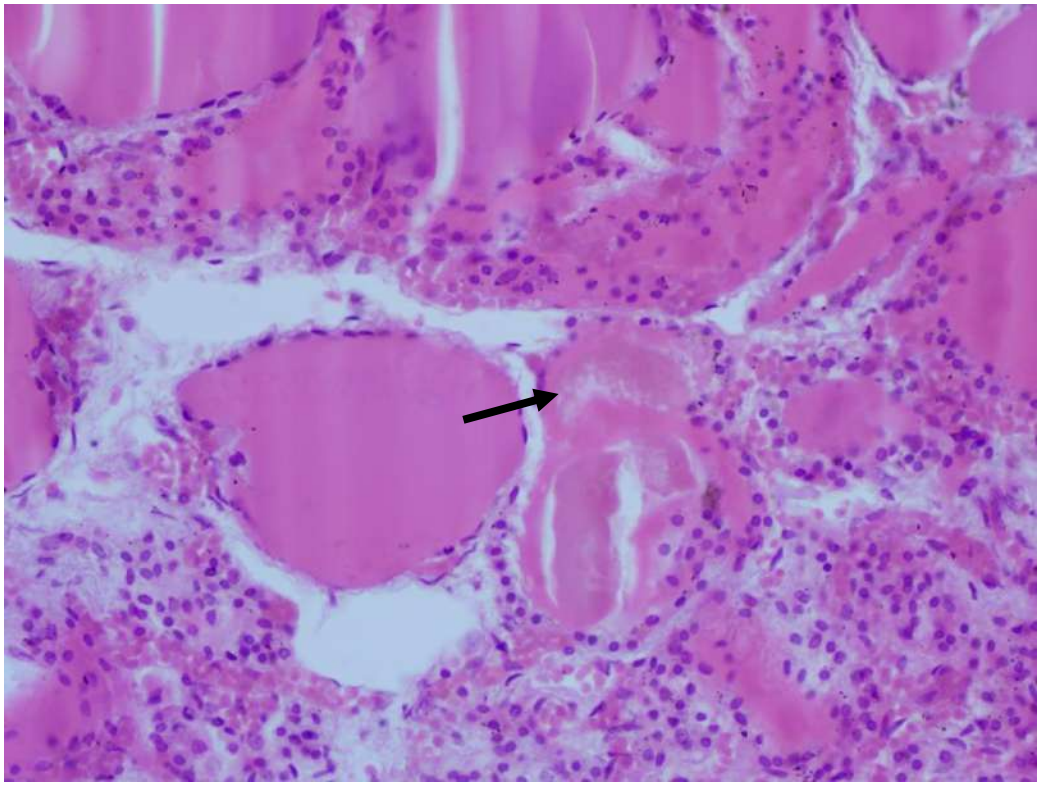


Figure 20 Microphotograph showing intrafollicular aggregates of RBC's (Arrow) (H&E: 40x).

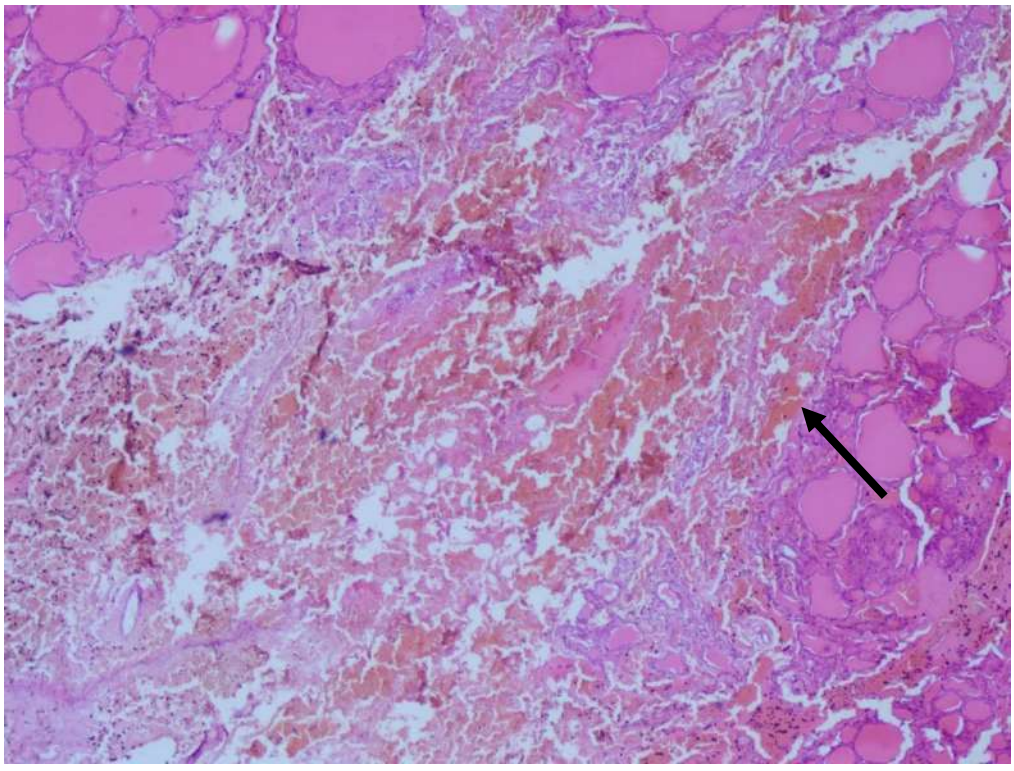


Figure 21 Microphotograph showing parenchymatous hemorrhage (Arrow) (H&E: 4x).

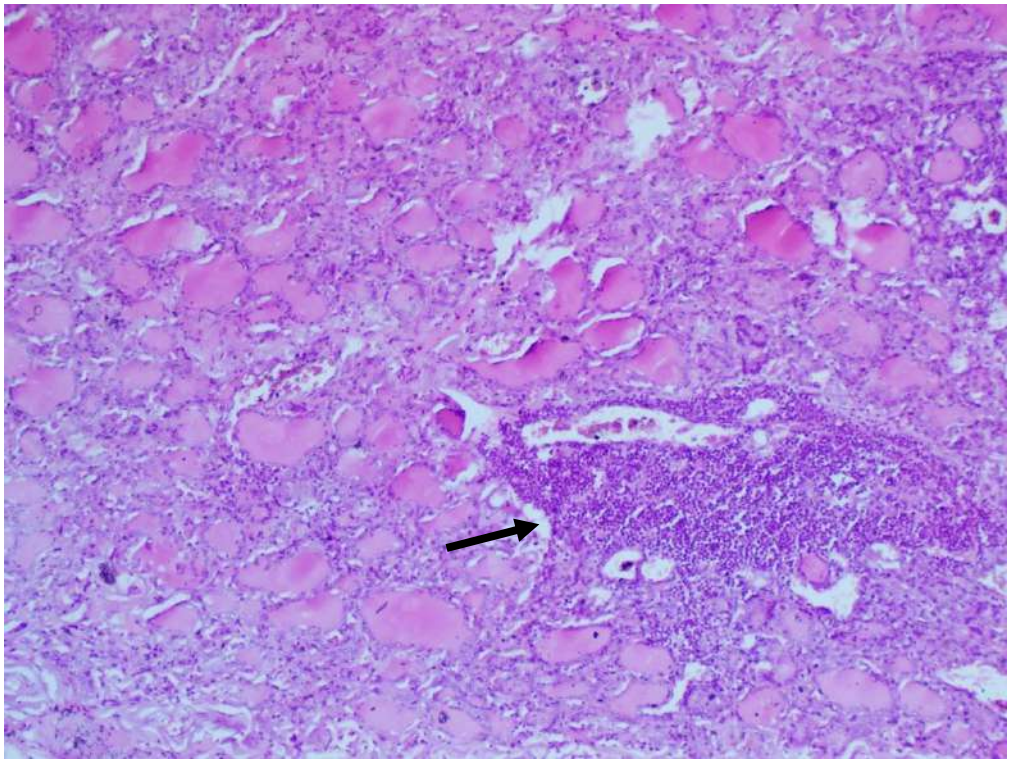
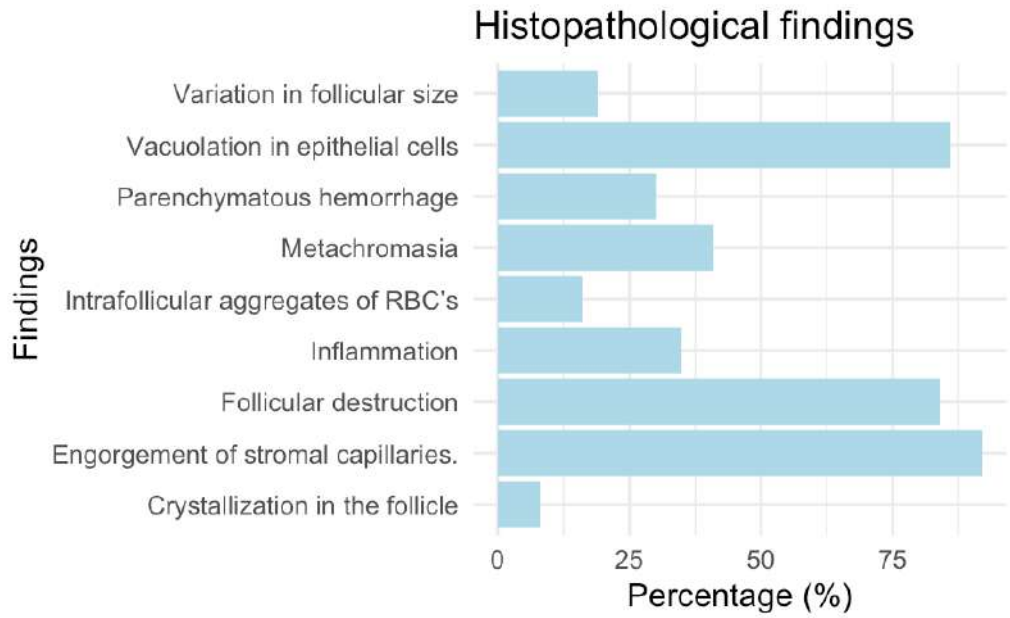


Figure 22 Microphotograph showing interstitial chronic inflammation (Arrow) (H&E: 4x).



Graph 1: Histopathological findings of Thyroid

HISTOPATHOLOGY OF THYROID IN RELATION TO TIME SINCE DEATH

Table 25: Histopathology of thyroid in relation to time since death.

Characteristic	Overall N = 37[1]	6hrs since death N = 9[1]	12hrs since death N = 18[1]	24hrs since death N = 10[1]	p-value[2]
Follicular destruction					0.3
Absent	6 (16%)	0 (0%)	3 (17%)	3 (30%)	
Present	31 (84%)	9 (100%)	15 (83%)	7 (70%)	
Vacuolation in epithelial cells					0.7
Absent	5 (14%)	2 (22%)	2 (11%)	1 (10%)	
Present	32 (86%)	7 (78%)	16 (89%)	9 (90%)	
Crystallization in the follicle					0.3
Absent	34 (92%)	9 (100%)	17 (94%)	8 (80%)	
Present	3 (8.1%)	0 (0%)	1 (5.6%)	2 (20%)	
Engorgement of stromal capillaries					0.6
Absent	3 (8.1%)	1 (11%)	2 (11%)	0 (0%)	
Present	34 (92%)	8 (89%)	16 (89%)	10 (100%)	
Parenchymatous hemorrhage					0.4
Absent	26 (70%)	7 (78%)	11 (61%)	8 (80%)	
Present	11 (30%)	2 (22%)	7 (39%)	2 (20%)	
Intrafollicular aggregates of RBCs					0.10
Absent	31 (84%)	6 (67%)	17 (94%)	8 (80%)	
Present	6 (16%)	3 (33%)	1 (5.6%)	2 (20%)	
Variation in follicular size					0.2
Absent	30 (81%)	7 (78%)	13 (72%)	10 (100%)	

Characteristic	Overall N = 37[1]	6hrs since death N = 9[1]	12hrs since death N = 18[1]	24hrs since death N = 10[1]	p-value[2]
Present	7 (19%)	2 (22%)	5 (28%)	0 (0%)	
Metachromasia					0.8
Absent	22 (59%)	5 (56%)	10 (56%)	7 (70%)	
Present	15 (41%)	4 (44%)	8 (44%)	3 (30%)	
Inflammation					0.6
Absent	24 (65%)	6 (67%)	13 (72%)	5 (50%)	
Acute inflammation	1 (2.7%)	0 (0%)	0 (0%)	1 (10%)	
Chronic inflammation	12 (32%)	3 (33%)	5 (28%)	4 (40%)	
[1] n (%)					
[2] Fisher's exact test					

The table details various histological characteristics observed at different times since death, categorized into 6 hours, 12 hours, and 24 hours post-mortem. Follicular destruction was present in 31 cases (84%) and absent in 6 cases (16%). Among the 6 hours since death group, all 9 individuals (100%) had follicular destruction. In the 12 hours since death group, 15 individuals (83%) had follicular destruction, with 3 individuals (17%) showing no destruction. For the 24 hours since death group, 7 individuals (70%) had follicular destruction, and 3 individuals (30%) did not. Vacuolation in epithelial cells was present in 32 cases (86%) and absent in 5 cases (14%). In the 6 hours group, 7 individuals (78%) had vacuolation, and 2 individuals (22%) did not. For the 12 hours group, 16 individuals (89%) had vacuolation, while 2 (11%) did not. In the 24 hours group, 9 individuals (90%) had vacuolation, and 1 (10%) did not. Crystallization in the follicle was absent in 34 cases (92%) and present in 3 cases (8.1%), with it being absent in all 9 individuals (100%) in the 6 hours group, 17 individuals (94%) in the 12 hours group, and 8 individuals (80%) in the 24 hours group. Engorgement of stromal capillaries was present in 34 cases (92%) and absent in 3 cases (8.1%), showing consistency across groups. Parenchymatous hemorrhage occurred in 11 cases (30%) and was absent in 26 cases (70%). Among the 6 hours group, hemorrhage was present in 2 individuals (22%) and absent in 7 (78%). In the 12 hours group, hemorrhage was present in 7 individuals (39%) and absent in 11 (61%). For the 24 hours group, hemorrhage was present in 2 individuals (20%) and absent in 8 (80%). Intrafollicular aggregates of RBCs were absent in 31 cases (84%) and present in 6 cases (16%), with differences

across groups. Variation in follicular size was present in 7 cases (19%) and absent in 30 cases (81%), showing different distributions among groups. Metachromasia was present in 15 cases (41%) and absent in 22 cases (59%), with similar distribution across groups. Inflammation was absent in 24 cases (65%) and present in 13 cases (35%), with chronic inflammation seen in 12 cases (32%) and acute inflammation in 1 case (2.7%), again with slight variations across groups.

BIOCHEMICAL MARKERS

Table 26: biochemical markers of thyroid in entire study group

Characteristic	Overall N = 76[1]	Case N = 37[1]	Control N = 39[1]	p-value[2]
T3 (ng/ml)	1.54 (1.59)	2.30 (1.98)	0.84 (0.53)	<0.001
Missing	5	3	2	
fT4 ng/dl	1.46 (0.99)	1.25 (0.32)	1.65 (1.31)	0.9
Missing	2	2	0	
TT4 (ug/dl)	7.12 (2.90)	8.35 (3.05)	5.89 (2.15)	<0.001
Missing	2	0	2	
TSH (uIU/ml)	2.9 (3.6)	2.0 (1.8)	3.8 (4.6)	0.4
Missing	2	0	2	
Thyroglobulin (ng/ml)	162 (145)	131 (137)	191 (147)	0.044
Missing	2	1	1	
[1] Mean (SD)				
[2] Wilcoxon rank sum test; Wilcoxon rank sum exact test				

In this table, From a total of 76 cases, the average T3 level was 1.54 ng/ml (SD 1.59). In the cases group, it was 2.30 ng/ml (SD 1.98), whereas in the control group, it was 0.84 ng/ml (SD 0.53), revealing a significant difference with a p-value of <0.001. For fT4 levels, the overall mean was 1.46 ng/dl (SD 0.99). Cases had a mean of 1.25 ng/dl (SD 0.32), and controls had 1.65 ng/dl (SD 1.31), with a p-value of 0.9. The Total T4 levels showed an overall mean of 7.12 ug/dl (SD 2.90), with cases at 8.35 ug/dl (SD 3.05) and controls at 5.89 ug/dl (SD 2.15), indicating a significant

difference with a p-value of <0.001. TSH levels had an overall average of 2.9 uIU/ml (SD 3.6), with cases at 2.0 uIU/ml (SD 1.8) and controls at 3.8 uIU/ml (SD 4.6), giving a p-value of 0.4. For Thyroglobulin, the overall mean was 162 ng/ml (SD 145). Cases had a mean of 131 ng/ml (SD 137), and controls had 191 ng/ml (SD 147), with a significant difference shown by a p-value of 0.044.

TSH

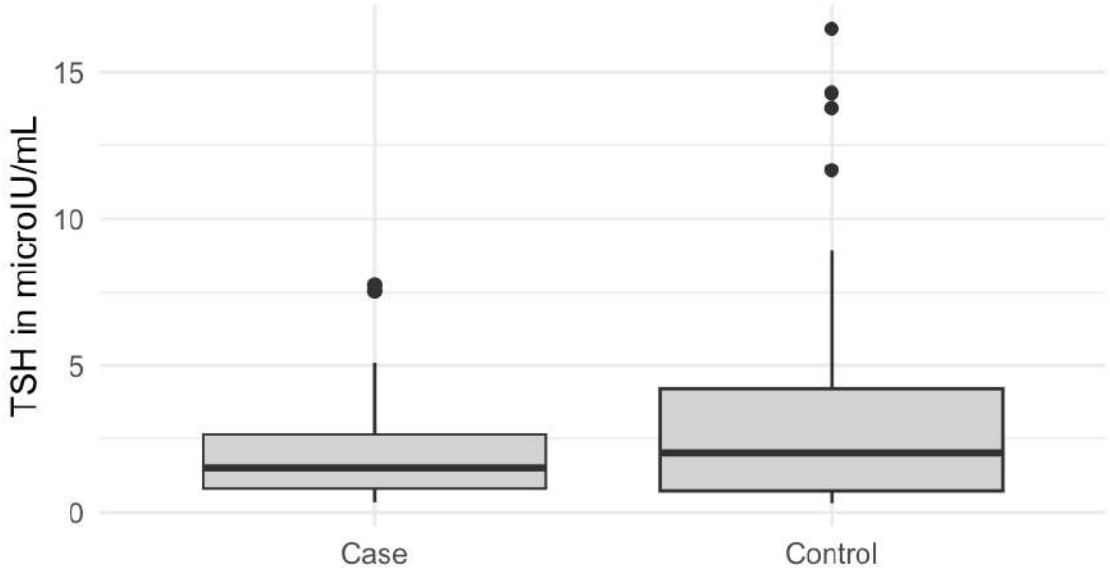
Table 27: Characteristics of TSH in entire study group

Characteristic	Overall N = 76[1]	Case N = 37[1]	Control N = 39[1]	p-value[2]
TSH category				**0.2**
High	**9 (12%)**	**2 (5.4%)**	**7 (19%)**	
Low	**2 (2.7%)**	**1 (2.7%)**	**1 (2.7%)**	
Normal	**63 (85%)**	**34 (92%)**	**29 (78%)**	
Missing	**2**	**0**	**2**	

[1] **n (%)**

[2] **Fisher's exact test; Pearson's Chi-squared test**

In this table, TSH levels were categorized for a total of 76 cases. Overall, 9 cases (12%) had high TSH levels, 2 cases (2.7%) had low levels, and 63 cases (85%) had normal levels. Specifically, within the cases group, 2 individuals (5.4%) had high TSH levels, 1 individual (2.7%) had low levels, and 34 individuals (92%) had normal levels. In the control group, 7 individuals (19%) had high levels, 1 individual (2.7%) had low levels, and 29 individuals (78%) had normal levels. The p-value was 0.2, indicating no significant difference between the groups.



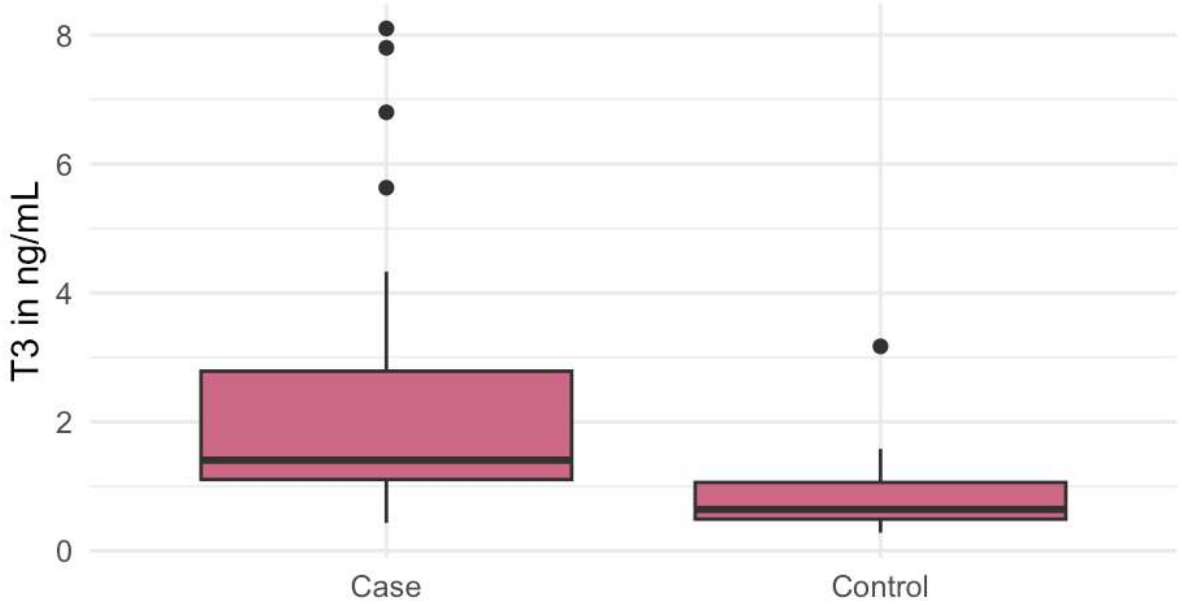
Graph 2: Whisker plot of values of TSH in case vs control

T3

Table 28: Characteristics of T3 in entire study group

Characteristic	Overall N = 76[1]	Case N = 37[1]	Control N = 39[1]	p-value[2]
T3 category				**<0.001**
High	**16 (23%)**	**15 (44%)**	**1 (2.7%)**	
Low	**21 (30%)**	**1 (2.9%)**	**20 (54%)**	
Normal	**34 (48%)**	**18 (53%)**	**16 (43%)**	
Missing	**5**	**3**	**2**	
[1] **n (%)**				
[2] **Fisher's exact test; Pearson's Chi-squared test**				

The table shows T3 levels categorized for the 76 cases studied. Overall, 16 cases (23%) had high T3 levels, 21 cases (30%) had low levels, and 34 cases (48%) had normal levels. Within the cases group, 15 individuals (44%) had high T3 levels, 1 individual (2.9%) had low levels, and 18 individuals (53%) had normal levels. Among the control group, 1 individual (2.7%) had high levels, 20 individuals (54%) had low levels, and 16 individuals (43%) had normal levels. The p-value was <0.001, indicating a significant difference between the groups.



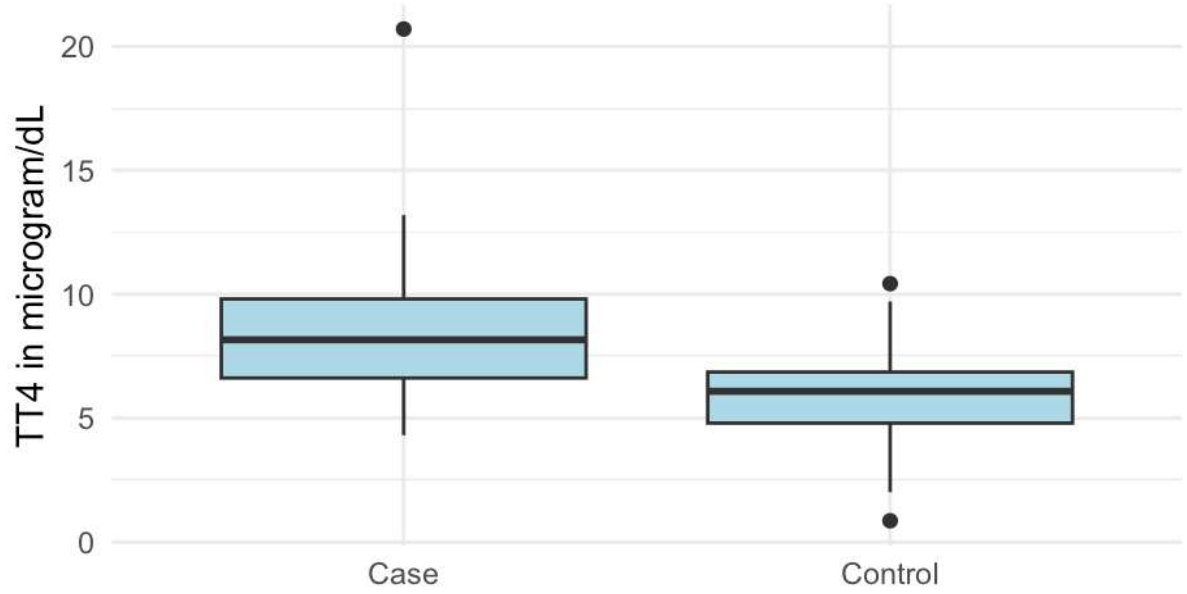
Graph 3: Whisker plot of values of T3 in case vs control

TOTAL T4

Table 29: Characteristics of TT4 in entire study group

Characteristic	Overall N = 76[1]	Case N = 37[1]	Control N = 39[1]	p-value[2]
TT4 category				**0.003**
High	**3 (4.1%)**	**3 (8.1%)**	**0 (0%)**	
Low	**29 (39%)**	**8 (22%)**	**21 (57%)**	
Normal	**42 (57%)**	**26 (70%)**	**16 (43%)**	
Missing	**2**	**0**	**2**	
[1] **n (%)**				
[2] **Fisher's exact test; Pearson's Chi-squared test**				

In this table, Total T4 levels were categorized for 76 cases. Overall, 3 cases (4.1%) had high Total T4 levels, 29 cases (39%) had low levels, and 42 cases (57%) had normal levels. Among the cases group, 3 individuals (8.1%) had high Total T4 levels, 8 individuals (22%) had low levels, and 26 individuals (70%) had normal levels. In the control group, no individuals had high Total T4 levels, 21 individuals (57%) had low levels, and 16 individuals (43%) had normal levels. The p-value was 0.003, indicating a significant difference between the groups.



Graph 4: Whisker plot of values of TT4 in case vs control

FT4

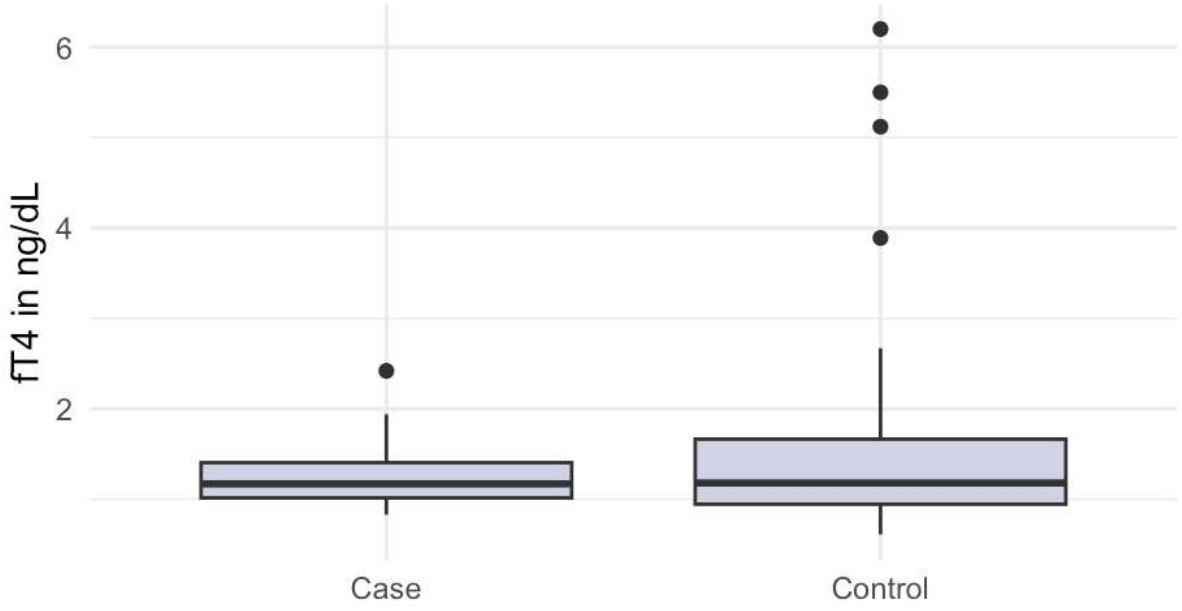
Table 30: Characteristics of FT4 in entire study group

Characteristic	Overall N = 76[1]	Case N = 37[1]	Control N = 39[1]	p-value[2]
fT4 category				0.10
High	11 (15%)	2 (5.7%)	9 (23%)	
Low	5 (6.8%)	2 (5.7%)	3 (7.7%)	
Normal	58 (78%)	31 (89%)	27 (69%)	
Missing	2	2	0	

[1] n (%)

[2] Fisher's exact test; Pearson's Chi-squared test

Out of a total of 76 cases, fT4 levels were categorized. In the overall group, 11 cases (15%) had high levels, 5 cases (6.8%) had low levels, and 58 cases (78%) had normal levels. Among the cases, 2 (5.7%) had high fT4 levels, 2 (5.7%) had low levels, and 31 (89%) had normal levels. In the control group, 9 (23%) had high levels, 3 (7.7%) had low levels, and 27 (69%) had normal levels. The p-value was 0.10, suggesting a trend but not a significant difference.



Graph 5: Whisker plot of values of FT4 in case vs control

THYROGLOBULIN

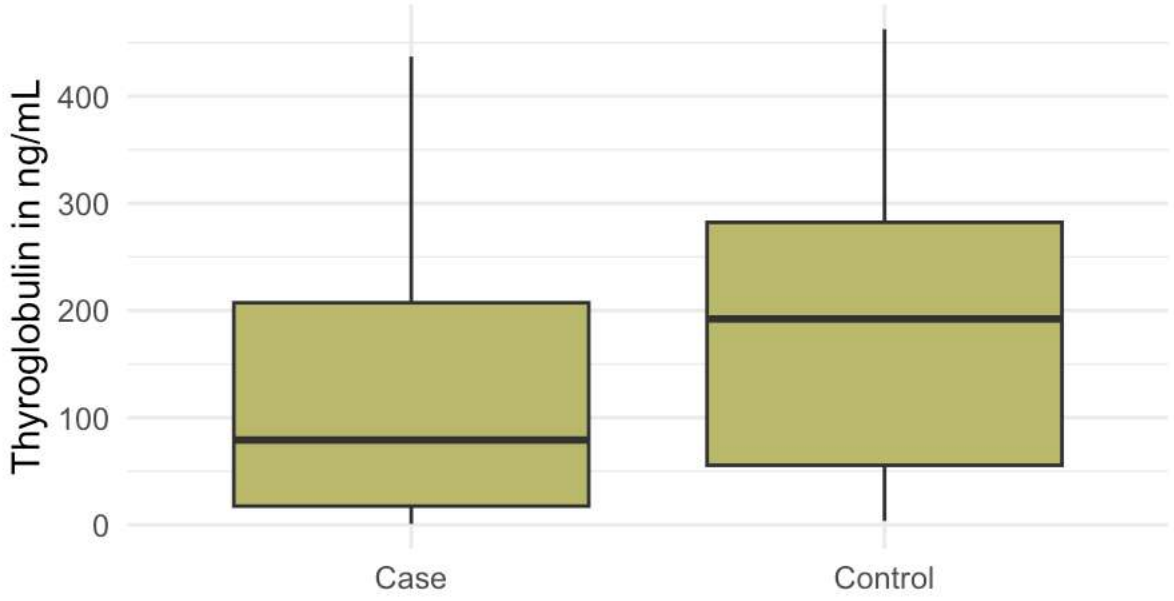
Table 31: Characteristics of Tg in entire study group

Characteristic	Overall N = 76[1]	Case N = 37[1]	Control N = 39[1]	p-value[2]
Thyroglobulin category				**0.2**
Abnormal	**45 (59%)**	**19 (51%)**	**26 (67%)**	
Normal	**31 (41%)**	**18 (49%)**	**13 (33%)**	

[1] **n (%)**

[2] **Fisher's exact test; Pearson's Chi-squared test**

Based on this table, Thyroglobulin levels were categorized for 76 cases. In the overall group, 45 cases (59%) had abnormal Thyroglobulin levels, while 31 cases (41%) had normal levels. Among the cases group, 19 individuals (51%) had abnormal levels, and 18 individuals (49%) had normal levels. In the control group, 26 individuals (67%) had abnormal levels, and 13 individuals (33%) had normal levels. The p-value was 0.2, indicating no statistically significant difference between the groups.



Graph 6: Whisker plot of values of Tg in case vs control

BIOCHEMICAL MARKERS IN RELATION TO TIME SINCE DEATH

Table 32: biochemical markers in relation to time since death

Characteristic	Overall N = 76[1]	6hrs N = 31[1]	12hrs N = 34[1]	24hrs N = 11[1]	p-value[2]
T3 (ng/ml)	1.08 (0.69 1.42)	1.02 (0.61 1.24)	1.08 (0.64 1.72)	1.22 (1.21, 2.15	0.10
Missing	12	6	4	2	
fT4 ng/dl	1.17 (1.01 1.43)	1.19 (0.93 1.59)	1.16 (1.02 1.29)	1.25 (1.01, 1.46	0.7
Missing	4	1	2	1	
TT4 (ug/dl)	6.72 (5.12 8.49)	6.16 (4.80 8.18)	6.62 (5.22 8.30)	8.33 (7.38 10.54)	0.015
Missing	2	1	1	0	
TSH (uIU/ml)	1.6 (0.8, 3.4)	1.1 (0.7, 2.4)	1.8 (0.9, 3.5)	1.7 (1.0, 3.6)	0.4
Missing	2	1	1	0	
Thyroglobulin (ng/ml)	128 (25, 263)	129 (30, 288)	107 (25, 216)	190 (17, 332)	0.9
Missing	2	2	0	0	

[1] Median (Q1, Q3)

[2] Kruskal-Wallis rank sum test

The table outlines various thyroid hormone levels measured at different intervals since death. The median T3 level overall was 1.08 ng/ml, with an interquartile range of 0.69 to 1.42. T3 levels were 1.02 ng/ml at 6 hours, 1.08 ng/ml at 12 hours, and 1.22 ng/ml at 24 hours, resulting in a p-value of 0.10. The fT4 levels showed a median of 1.17 ng/dl (interquartile range: 1.01 to 1.43) overall, with 1.19 ng/dl at 6 hours, 1.16 ng/dl at 12 hours, and 1.25 ng/dl at 24 hours, having a p-

value of 0.7. For Total T4, the overall median was 6.72 ug/dl (interquartile range: 5.12 to 8.49), with 6.16 ug/dl at 6 hours, 6.62 ug/dl at 12 hours, and 8.33 ug/dl at 24 hours, which showed a significant difference with a p-value of 0.015. The TSH levels had a median of 1.6 uIU/ml (interquartile range: 0.8 to 3.4) overall, with 1.1 uIU/ml at 6 hours, 1.8 uIU/ml at 12 hours, and 1.7 uIU/ml at 24 hours, with a p-value of 0.4. Lastly, the Thyroglobulin levels had a median of 128 (interquartile range: 25 to 263) overall, with 129 at 6 hours, 107 at 12 hours, and 190 at 24 hours, showing no significant difference with a p-value of 0.9.

BIOCHEMICAL MARKERS IN RELATION TO CHARACTERISTICS OF HANGING

T3

Table 33: T3 in relation to time since death characteristics of hanging

Characteristic vs T3 values	**Overall N = 34[1]**	**High N = 15[1]**	**Low N = 1[1]**	**Normal N = 18[1]**	**P-value[2]**
Ligature material type					>0.9
Hard	10 (31%)	5 (36%)	0 (0%)	5 (29%)	
Soft	22 (69%)	9 (64%)	1 (100%)	12 (71%)	
Unknown	2	1	0	1	
Type of hanging					0.6
Complete	31 (91%)	13 (87%)	1 (100%)	17 (94%)	
Partial	3 (8.8%)	2 (13%)	0 (0%)	1 (5.6%)	
Ligature mark impression					>0.9
Faint	3 (8.8%)	1 (6.7%)	0 (0%)	2 (11%)	
Grooved	10 (29%)	4 (27%)	0 (0%)	6 (33%)	
Prominent	21 (62%)	10 (67%)	1 (100%)	10 (56%)	
Knot position					0.7
Anterior	1 (2.9%)	1 (6.7%)	0 (0%)	0 (0%)	
Lateral	8 (24%)	4 (27%)	0 (0%)	4 (22%)	
Posterior	25 (74%)	10 (67%)	1 (100%)	14 (78%)	

Characteristic vs T3 values	Overall N = 34[1]	High N = 15[1]	Low N = 1[1]	Normal N = 18[1]	P-value[2]
[1] n (%); Median (Q1, Q3)					
[2] Fisher's exact test; Kruskal-Wallis rank sum test					

Ligature Material Type (N = 32):

- Total: 22 cases (69%) used soft material, 10 cases (31%) used hard material.
- Normal T3 (N = 17): 12 cases (71%) used soft material, 5 cases (29%) used hard material.
- Low T3 (N = 1): 1 case (100%) used soft material, 0 cases used hard material.
- High T3 (N = 14): 9 cases (64%) used soft material, 5 cases (36%) used hard material.
- p-value: >0.9

Type of Hanging (N = 34):

- Total: 31 cases (91%) were complete hanging, 3 cases (8.8%) were partial hanging.
- Normal T3 (N = 18): 17 cases (94%) were complete hanging, 1 case (5.6%) was partial.
- Low T3 (N = 1): 1 case (100%) was complete hanging.
- High T3 (N = 15): 13 cases (87%) were complete hanging, 2 cases (13%) were partial.
- p-value: 0.6

Ligature Mark Impression (N = 34):

- Total: 21 cases (62%) had prominent impressions, 10 cases (29%) were grooved, 3 cases (8.8%) were faint.
- Normal T3 (N = 18): 10 cases (56%) had prominent impressions, 6 cases (33%) were grooved, 2 cases (11%) were faint.
- Low T3 (N = 1): 1 case (100%) had prominent impressions.
- High T3 (N = 15): 10 cases (67%) had prominent impressions, 4 cases (27%) were grooved, 1 case (6.7%) was faint.
- p-value: >0.9

Knot Position (N = 34):

- Total: 25 cases (74%) had posterior knots, 8 cases (24%) had lateral knots, 1 case (2.9%) had an anterior knot.

- Normal T3 (N = 18): 14 cases (78%) had posterior knots, 4 cases (22%) had lateral knots, 0 cases had anterior knots.
- Low T3 (N = 1): 1 case (100%) had a posterior knot.
- High T3 (N = 15): 10 cases (67%) had posterior knots, 4 cases (27%) had lateral knots, 1 case (6.7%) had an anterior knot.
- p-value: 0.7

TOTAL T4

Table 34: TT4 in relation to time since death characteristics of hanging

Characteristic	Overall N = 37[1]	High N = 3[1]	Low N = 8[1]	Normal N = 26[1]	P-value[2]
Ligature material type					0.2
Hard	11 (31%)	2 (67%)	3 (43%)	6 (24%)	
Soft	24 (69%)	1 (33%)	4 (57%)	19 (76%)	
Unknown	2	0	1	1	
Type of hanging					0.3
Complete	33 (89%)	2 (67%)	8 (100%)	23 (88%)	
Partial	4 (11%)	1 (33%)	0 (0%)	3 (12%)	
Ligature mark impression					0.6
Faint	3 (8.1%)	0 (0%)	0 (0%)	3 (12%)	
Grooved	11 (30%)	2 (67%)	2 (25%)	7 (27%)	
Prominent	23 (62%)	1 (33%)	6 (75%)	16 (62%)	
Knot position					0.5
Anterior	1 (2.7%)	0 (0%)	0 (0%)	1 (3.8%)	
Lateral	9 (24%)	2 (67%)	2 (25%)	5 (19%)	
Posterior	27 (73%)	1 (33%)	6 (75%)	20 (77%)	

[1] **n (%); Median (Q1, Q3)**

[2] **Fisher's exact test; Kruskal-Wallis rank sum test**

Ligature Material Type (N = 35):

- Total: 24 cases (69%) used soft material, 11 cases (31%) used hard material.
- Normal TT4 (N = 25): 19 cases (76%) used soft material, 6 cases (24%) used hard material.
- Low TT4 (N = 7): 4 cases (57%) used soft material, 3 cases (43%) used hard material.

- High TT4 (N = 3): 1 case (33%) used soft material, 2 cases (67%) used hard material.
- p-value: 0.2

Type of Hanging (N = 37):

- Total: 33 cases (89%) were complete hanging, 4 cases (11%) were partial hanging.
- Normal TT4 (N = 26): 23 cases (88%) were complete hanging, 3 cases (12%) were partial.
- Low TT4 (N = 8): All 8 cases (100%) were complete hanging.
- High TT4 (N = 3): 2 cases (67%) were complete hanging, 1 case (33%) was partial.
- p-value: 0.3

Ligature Mark Impression (N = 37):

- Total: 33 cases (89%) were complete hanging, 4 cases (11%) were partial hanging.
- Normal TT4 (N = 26): 23 cases (88%) were complete hanging, 3 cases (12%) were partial.
- Low TT4 (N = 8): All 8 cases (100%) were complete hanging.
- High TT4 (N = 3): 2 cases (67%) were complete hanging, 1 case (33%) was partial.
- p-value: 0.3

Knot Position (N = 37):

- Total: 27 cases (73%) had posterior knots, 9 cases (24%) had lateral knots, 1 case (2.7%) had an anterior knot.
- Normal TT4 (N = 26): 20 cases (77%) had posterior knots, 5 cases (19%) had lateral knots, 1 case (3.8%) had an anterior knot.
- Low TT4 (N = 8): 6 cases (75%) had posterior knots, 2 cases (25%) had lateral knots.
- High TT4 (N = 3): 1 case (33%) had posterior knots, 2 cases (67%) had lateral knots.
- p-value: 0.5

FT4

Table 35: FT4 in relation to time since death characteristics of hanging

Characteristic	Overall N = 35[1]	High N = 2[1]	Low N = 2[1]	Normal N = 31[1]	P-value[2]
Ligature material type					0.10
Hard	11 (33%)	1 (50%)	2 (100%)	8 (28%)	
Soft	22 (67%)	1 (50%)	0 (0%)	21 (72%)	
Unknown	2	0	0	2	
Type of hanging					>0.9
Complete	31 (89%)	2 (100%)	2 (100%)	27 (87%)	
Partial	4 (11%)	0 (0%)	0 (0%)	4 (13%)	
Ligature mark impression					>0.9
Faint	3 (8.6%)	0 (0%)	0 (0%)	3 (9.7%)	
Grooved	11 (31%)	1 (50%)	1 (50%)	9 (29%)	
Prominent	21 (60%)	1 (50%)	1 (50%)	19 (61%)	
Knot position					0.8
Anterior	1 (2.9%)	0 (0%)	0 (0%)	1 (3.2%)	
Lateral	9 (26%)	0 (0%)	1 (50%)	8 (26%)	
Posterior	25 (71%)	2 (100%)	1 (50%)	22 (71%)	
[1] n (%); Median (Q1, Q3)					
[2] Fisher's exact test; Kruskal-Wallis rank sum test					

Ligature Material Type (N = 33):

- Total: 22 cases (67%) used soft material, 11 cases (33%) used hard material.
- Normal FT4 (N = 29): 21 cases (72%) used soft material, 8 cases (28%) used hard material.
- Low FT4 (N = 2): Both cases (100%) used hard material.

- High FT4 (N = 2): Both cases (50%) used soft and hard material equally.
- p-value: 0.10

Type of Hanging (N = 35):

- Total: 31 cases (89%) were complete hanging, 4 cases (11%) were partial hanging.
- Normal FT4 (N = 31): 27 cases (87%) were complete hanging, 4 cases (13%) were partial.
- Low FT4 (N = 2): Both cases (100%) were complete hanging.
- High FT4 (N = 2): Both cases (100%) were complete hanging.
- p-value: >0.9

Ligature Mark Impression (N = 35):

- Total: 21 cases (60%) had prominent impressions, 11 cases (31%) were grooved, 3 cases (8.6%) were faint.
- Normal FT4 (N = 31): 19 cases (61%) had prominent impressions, 9 cases (29%) were grooved, 3 cases (9.7%) were faint.
- Low FT4 (N = 2): Both cases (50%) had prominent and grooved impressions.
- High FT4 (N = 2): Both cases (50%) had prominent and grooved impressions.
- p-value: >0.9

Knot Position (N = 35):

- Total: 25 cases (71%) had posterior knots, 9 cases (26%) had lateral knots, 1 case (2.9%) had an anterior knot.
- Normal FT4 (N = 31): 22 cases (71%) had posterior knots, 8 cases (26%) had lateral knots, 1 case (3.2%) had an anterior knot.
- Low FT4 (N = 2): Both cases (50%) had posterior and lateral knots.
- High FT4 (N = 2): Both cases (100%) had posterior knots.
- p-value: 0.8

THYROGLOBULIN

Table 36: Tg in relation to time since death characteristics of hanging

Characteristic	Overall N = 37[1]	Abnormal N = 19[1]	Normal N = 18[1]	P-value[2]
Ligature material type				0.2
Hard	11 (31%)	7 (41%)	4 (22%)	
Soft	24 (69%)	10 (59%)	14 (78%)	
Unknown	2	2	0	
Type of hanging				0.046
Complete	33 (89%)	19 (100%)	14 (78%)	
Partial	4 (11%)	0 (0%)	4 (22%)	
Ligature mark impression				0.029
Faint	3 (8.1%)	2 (11%)	1 (5.6%)	
Grooved	11 (30%)	9 (47%)	2 (11%)	
Prominent	23 (62%)	8 (42%)	15 (83%)	
Knot position				>0.9
Anterior	1 (2.7%)	1 (5.3%)	0 (0%)	
Lateral	9 (24%)	5 (26%)	4 (22%)	
Posterior	27 (73%)	13 (68%)	14 (78%)	

[1] n (%); Median (Q1, Q3)

[2] Fisher's exact test; Pearson's Chi-squared test; Wilcoxon rank sum test

Ligature Material Type (N = 35):

- Total: 24 cases (69%) used soft material; 11 cases (31%) used hard material.
- Normal: 14 cases (78%) used soft material, 4 cases (22%) used hard material.
- Abnormal: 10 cases (59%) used soft material, 7 cases (41%) used hard material.
- p-value: 0.2

Type of Hanging (N = 37):

- Total: 33 cases (89%) were complete hanging, 4 cases (11%) were partial hanging.
- Normal: 14 cases (78%) were complete hanging, 4 cases (22%) were partial.
- Abnormal: All 19 cases (100%) were complete hanging.
- p-value: 0.046

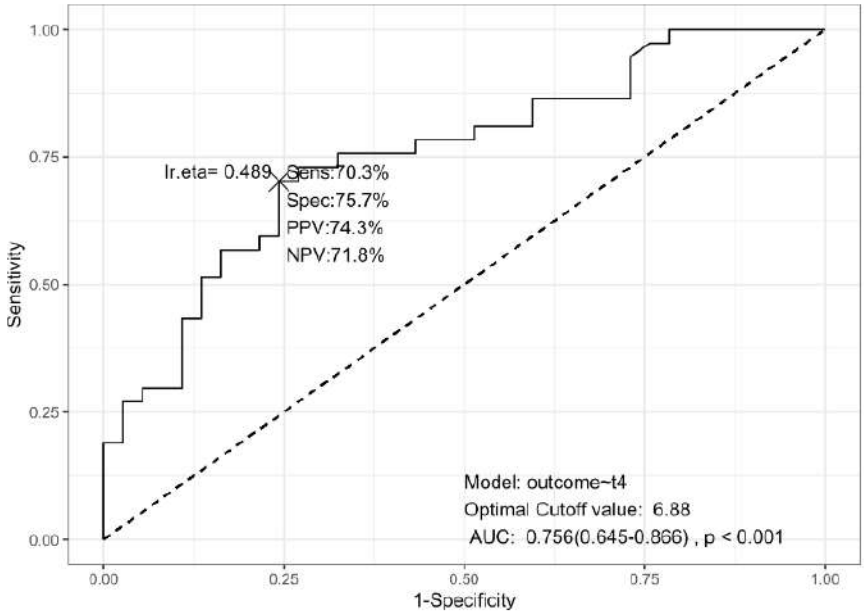
Ligature Mark Impression (N = 37):

- Total: 23 cases (62%) had prominent impressions, 11 cases (30%) were grooved, 3 cases (8.1%) were faint.
- Normal: 15 cases (83%) had prominent impressions, 2 cases (11%) were grooved, 1 case (5.6%) was faint.
- Abnormal: 8 cases (42%) had prominent impressions, 9 cases (47%) were grooved, 2 cases (11%) were faint.
- p-value: 0.029
- **Knot Position (N = 37):**
- Total: 27 cases (73%) had posterior knots, 9 cases (24%) had lateral knots, 1 case (2.7%) had an anterior knot.
- Normal: 14 cases (78%) had posterior knots, 4 cases (22%) had lateral knots, 0 cases had anterior knots.
- Abnormal: 13 cases (68%) had posterior knots, 5 cases (26%) had lateral knots, 1 case (5.3%) had an anterior knot.
- p-value: >0.9

PREDICTION OF HANGING IN AUTOPSIES USING ROC CURVE OF BIOCHEMICAL MARKERS

Receiver operating characteristic (ROC) curve analysis and associated statistics were applied to establish diagnostic accuracy and optimal cut-off levels for discriminating hanging from non-hanging cases.

TOTAL T4



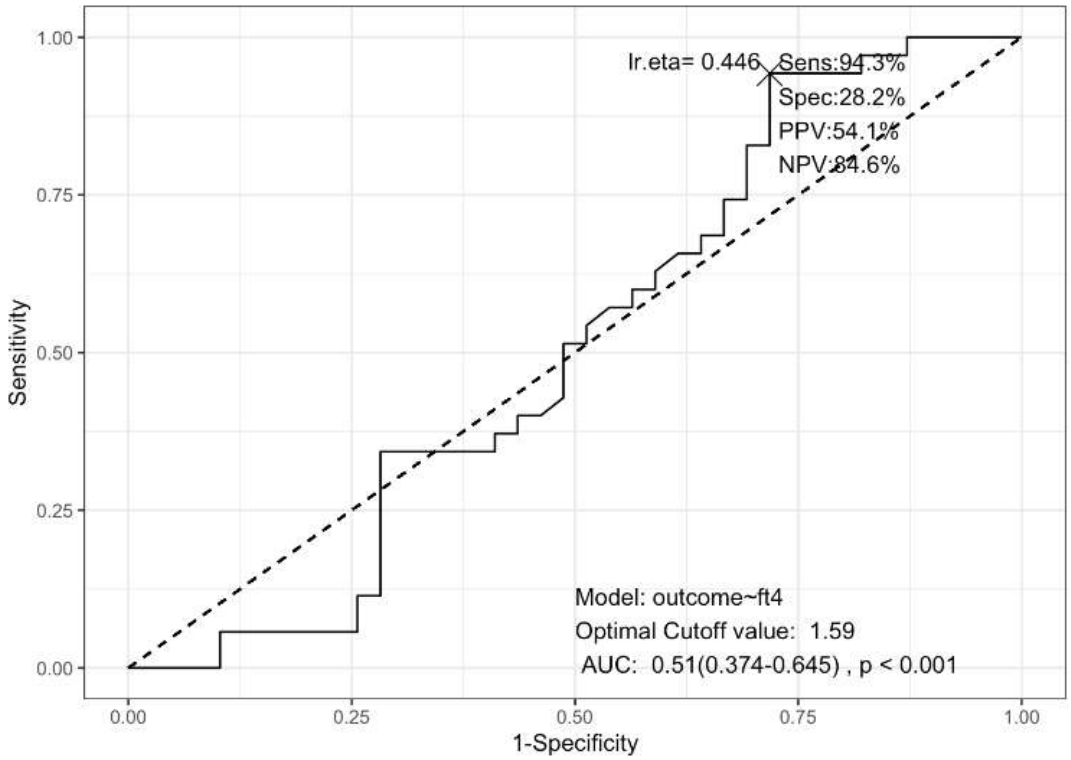
Graph 7: ROC of values of TT4 in case vs control

Table 37: Test values of TT4 as per ROC curve for prediction of hanging in autopsies

Test values of TT4 as per ROC curve for prediction of hanging in autopsies		
Cutoff	**Specificity**	**Sensitivity**
6.88	75.7 %	70.3%

The ROC curve analysis for TT4 levels in predicting hanging in autopsies has identified a cutoff value of 6.88 µg/dl. At this cutoff, the specificity is 75.7% and sensitivity is 70.3%.

FT4



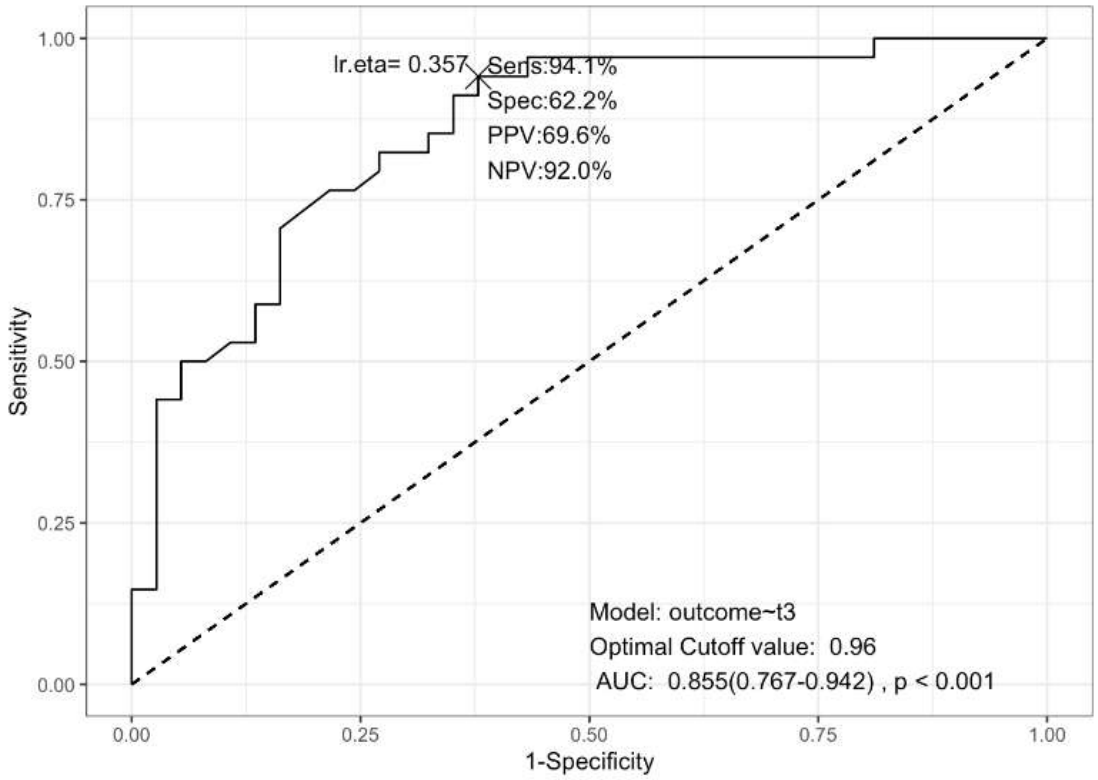
Graph 8: ROC of values of FT4 in case vs control

Table 38: Test values of FT4 as per ROC curve for prediction of hanging in autopsies

Test values of FT4 as per ROC curve for prediction of hanging in autopsies		
Cutoff	**Specificity**	**Sensitivity**
1.59	28.2	94.3

The ROC curve analysis for FT4 levels in predicting hanging in autopsies identified a cutoff value of 1.59 ng/dl. At this cutoff, the specificity is 28.2% and sensitivity is 94.3%.

T3



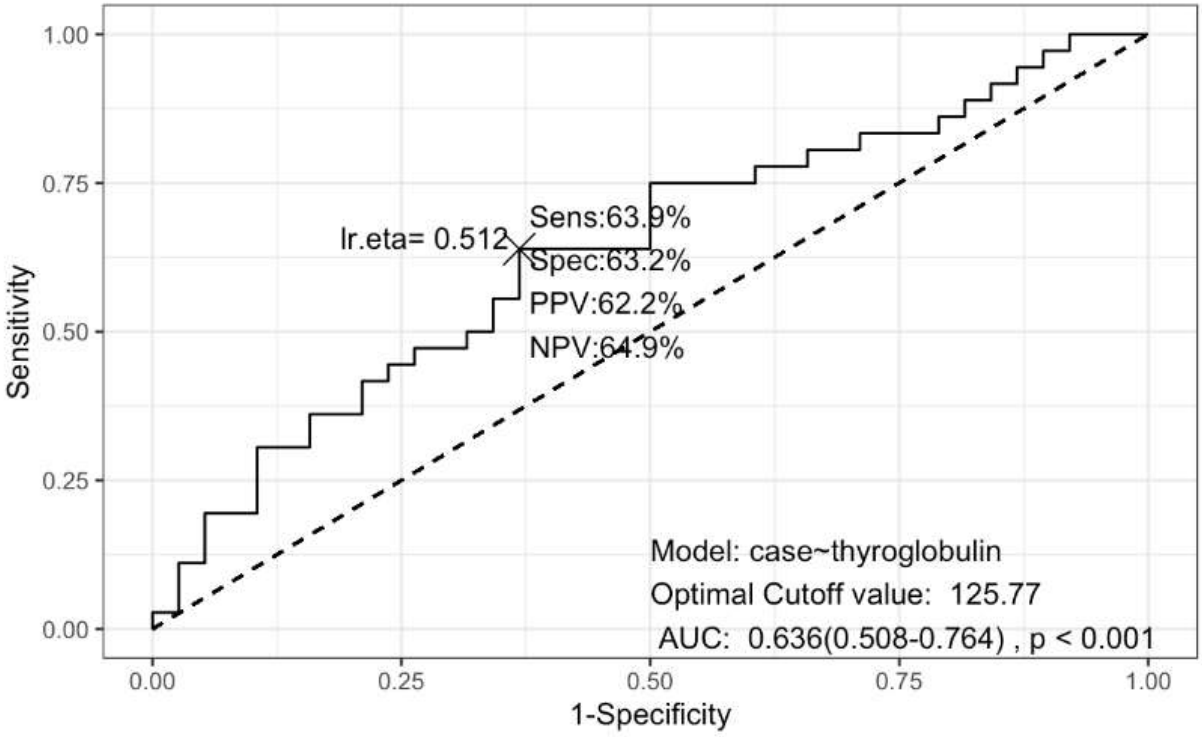
Graph 9: ROC of values of T3 in case vs control

Table 39: Test values of T3 as per ROC curve for prediction of hanging in autopsies

Test values of T3 as per ROC curve for prediction of hanging in autopsies		
Cutoff	**Specificity**	**Sensitivity**
096	62.2 %	94.1 %

The ROC curve analysis for T3 levels in predicting hanging in autopsies identified a cutoff value of 0.96 ng/ml. At this cutoff, the specificity is 62.2% and the sensitivity is 94.1%.

THYROGLOBULIN



Graph 10: ROC of values of Tg in case vs control

Table 40: Test values of Tg as per ROC curve for prediction of hanging in autopsies

Test values of Thyroglobulin as per ROC curve for prediction of hanging in autopsies		
Cutoff	**Specificity**	**Sensitivity**
125.77	63.2 %	63.9 %

The ROC curve analysis for Thyroglobulin levels in predicting hanging in autopsies identified a cutoff value of 125.77 ng/ml. At this cutoff, the specificity is 63.2% and the sensitivity is 63.9%.

BIOCHEMICAL MARKERS IN RELATION TO HISTOPATHOLOGY

Thyroglobulin

Table 41: Tg in relation to histopathology

Characteristic	**Overall** N = 37[1]	**Abnormal** N = 19[1]	**Normal** N = 18[1]	**p-value**[2]
Follicular destruction				0.7
Absent	6 (16%)	4 (21%)	2 (11%)	
Present	31 (84%)	15 (79%)	16 (89%)	
Vacuolation in epithelial cells				>0.9
Absent	5 (14%)	3 (16%)	2 (11%)	
Present	32 (86%)	16 (84%)	16 (89%)	
Crystallization in the follicle				0.11
Absent	34 (92%)	19 (100%)	15 (83%)	
Present	3 (8.1%)	0 (0%)	3 (17%)	
Engorgement of stromal capillaries.				>0.9
Absent	3 (8.1%)	2 (11%)	1 (5.6%)	
Present	34 (92%)	17 (89%)	17 (94%)	
Parenchymatous hemorrhage				>0.9
Absent	26 (70%)	13 (68%)	13 (72%)	
Present	11 (30%)	6 (32%)	5 (28%)	

Characteristic	Overall N = 37[1]	Abnormal N = 19[1]	Normal N = 18[1]	p-value[2]
Intrafollicular aggregates of RBC's				0.7
Absent	31 (84%)	15 (79%)	16 (89%)	
Present	6 (16%)	4 (21%)	2 (11%)	
Variation in follicular size				0.2
Absent	30 (81%)	17 (89%)	13 (72%)	
Present	7 (19%)	2 (11%)	5 (28%)	
Metachromasia				0.12
Absent	22 (59%)	9 (47%)	13 (72%)	
Present	15 (41%)	10 (53%)	5 (28%)	
Inflammation				0.4
Absent	24 (65%)	14 (74%)	10 (56%)	
Acute inflammation	1 (2.7%)	0 (0%)	1 (5.6%)	
Chronic inflammation	12 (32%)	5 (26%)	7 (39%)	

[1] n (%); Median (Q1, Q3)

[2] Fisher's exact test; Pearson's Chi-squared test; Wilcoxon rank sum exact test

In this table, histopathological findings were categorized for 37 cases. Follicular destruction was present in 31 cases (84%) and absent in 6 cases (16%). Within the abnormal thyroglobulin group, 15 individuals (79%) had follicular destruction, whereas 4 individuals (21%) did not. Similarly, in the normal thyroglobulin group, 16 individuals (89%) had follicular destruction, with 2 individuals (11%) showing no destruction. For vacuolation in epithelial cells, it was present in 32 cases (86%) and absent in 5 cases (14%). Among those with abnormal thyroglobulin levels, vacuolation was seen in 16 individuals (84%) and absent in 3 (16%), while in the normal group,

it was present in 16 individuals (89%) and absent in 2 (11%). Crystallization in the follicle was absent in 34 cases (92%) and present in 3 cases (8.1%), all of whom were in the normal group. Engorgement of stromal capillaries was seen in 34 cases (92%) and absent in 3 cases (8.1%), with similar distributions between the abnormal and normal thyroglobulin groups. Parenchymatous hemorrhage occurred in 11 cases (30%) and was absent in 26 cases (70%). Among those with abnormal thyroglobulin, hemorrhage was present in 6 individuals (32%) and absent in 13 (68%), and in the normal group, it was seen in 5 individuals (28%) and absent in 13 (72%). Intrafollicular aggregates of RBC's were absent in 31 cases (84%) and present in 6 cases (16%), with slight variations between the groups. Variation in follicular size was present in 7 cases (19%) and absent in 30 cases (81%), with higher absence in the abnormal thyroglobulin group. Metachromasia was absent in 22 cases (59%) and present in 15 cases (41%), with a higher presence in the abnormal group. Finally, inflammation was mostly absent (24 cases, 65%), with chronic inflammation seen in 12 cases (32%) and acute inflammation in 1 case (2.7%), again with slight variations between groups. None of the characteristics showed significant differences based on the p-values.

T3

Table 42: T3 in relation to histopathology

Characteristic	**Overall** N = 34[1]	**High** N = 15[1]	**Low** N = 1[1]	**Normal** N = 18[1]	**p-value**[2]
Follicular destruction					>0.9
Absent	5 (15%)	2 (13%)	0 (0%)	3 (17%)	
Present	29 (85%)	13 (87%)	1 (100%)	15 (83%)	
Vacuolation in epithelial cells					0.7
Absent	5 (15%)	3 (20%)	0 (0%)	2 (11%)	
Present	29 (85%)	12 (80%)	1 (100%)	16 (89%)	
Crystallization in the follicle					>0.9
Absent	31 (91%)	14 (93%)	1 (100%)	16 (89%)	
Present	3 (8.8%)	1 (6.7%)	0 (0%)	2 (11%)	
Engorgement of stromal capillaries.					>0.9

Characteristic	**Overall** N = 34[1]	**High** N = 15[1]	**Low** N = 1[1]	**Normal** N = 18[1]	**p-value**[2]
Absent	3 (8.8%)	1 (6.7%)	0 (0%)	2 (11%)	
Present	31 (91%)	14 (93%)	1 (100%)	16 (89%)	
Parenchymatous hemorrhage					0.6
Absent	24 (71%)	9 (60%)	1 (100%)	14 (78%)	
Present	10 (29%)	6 (40%)	0 (0%)	4 (22%)	
Intrafollicular aggregates of RBC's					0.7
Absent	29 (85%)	12 (80%)	1 (100%)	16 (89%)	
Present	5 (15%)	3 (20%)	0 (0%)	2 (11%)	
Variation in follicular size					0.7
Absent	28 (82%)	13 (87%)	1 (100%)	14 (78%)	
Present	6 (18%)	2 (13%)	0 (0%)	4 (22%)	
Metachromasia					>0.9
Absent	21 (62%)	9 (60%)	1 (100%)	11 (61%)	
Present	13 (38%)	6 (40%)	0 (0%)	7 (39%)	
Inflammation					0.7
Absent	22 (65%)	11 (73%)	1 (100%)	10 (56%)	
Acute inflammation	1 (2.9%)	0 (0%)	0 (0%)	1 (5.6%)	
Chronic inflammation	11 (32%)	4 (27%)	0 (0%)	7 (39%)	
[1] n (%)					
[2] Fisher's exact test					

In this table, histopathological findings were grouped based on T3 levels for 34 cases.

Follicular Destruction:

- 5 cases (15%) showed no destruction, 29 cases (85%) did.

- High T3: 13 individuals (87%) had destruction, 2 (13%) did not.
- Low T3: 1 individual (100%) had destruction.
- Normal T3: 15 individuals (83%) had destruction, 3 (17%) did not.
- p-value: >0.9, no significant difference.

Vacuolation in Epithelial Cells:

- 5 cases (15%) had no vacuolation, 29 cases (85%) did.
- High T3: 12 individuals (80%) had vacuolation, 3 (20%) did not.
- Low T3: 1 individual (100%) had vacuolation.
- Normal T3: 16 individuals (89%) had vacuolation, 2 (11%) did not.
- p-value: 0.7, no significant difference.

Crystallization in the Follicle:

- 31 cases (91%) had no crystallization, 3 cases (8.8%) did.
- High T3: 1 individual (6.7%) had crystallization, 14 (93%) did not.
- Low T3: no individuals had crystallization.
- Normal T3: 2 individuals (11%) had crystallization, 16 (89%) did not.
- p-value: >0.9, no significant difference.

Engorgement of Stromal Capillaries:

- 3 cases (8.8%) had no engorgement, 31 cases (91%) did.
- High T3: 1 individual (6.7%) had no engorgement, 14 (93%) did.
- Low T3: 1 individual (100%) had engorgement.
- Normal T3: 2 individuals (11%) had no engorgement, 16 (89%) did.
- p-value: >0.9, no significant difference.

Parenchymatous Hemorrhage:

- 24 cases (71%) had no hemorrhage, 10 cases (29%) did.
- High T3: 9 individuals (60%) had no hemorrhage, 6 (40%) did.
- Low T3: 1 individual (100%) had no hemorrhage.
- Normal T3: 14 individuals (78%) had no hemorrhage, 4 (22%) did.
- p-value: 0.6, no significant difference.

Intrafollicular Aggregates of RBC's:

- 29 cases (85%) had no aggregates, 5 cases (15%) did.
- High T3: 12 individuals (80%) had no aggregates, 3 (20%) did.
- Low T3: 1 individual (100%) had no aggregates.
- Normal T3: 16 individuals (89%) had no aggregates, 2 (11%) did.
- p-value: 0.7, no significant difference.

Variation in Follicular Size:

- 28 cases (82%) had no variation, 6 cases (18%) did.
- High T3: 13 individuals (87%) had no variation, 2 (13%) did.
- Low T3: 1 individual (100%) had no variation.
- Normal T3: 14 individuals (78%) had no variation, 4 (22%) did.
- p-value: 0.7, no significant difference.

Metachromasia:

- 21 cases (62%) had no metachromasia, 13 cases (38%) did.
- High T3: 9 individuals (60%) had no metachromasia, 6 (40%) did.
- Low T3: 1 individual (100%) had no metachromasia.
- Normal T3: 11 individuals (61%) had no metachromasia, 7 (39%) did.
- p-value: >0.9, no significant difference.

Inflammation:

- 22 cases (65%) had no inflammation, 11 cases (32%) had chronic inflammation, 1 case (2.9%) had acute inflammation.
- High T3: 11 individuals (73%) had no inflammation, 4 (27%) had chronic inflammation.
- Low T3: 1 individual (100%) had no inflammation.
- Normal T3: 10 individuals (56%) had no inflammation, 7 (39%) had chronic inflammation, 1 (5.6%) had acute inflammation.
- p-value: 0.7, no significant difference.

TOTAL T4

Table 43: TT4 in relation to histopathology

Characteristic	**Overall** N = 37[1]	**High** N = 3[1]	**Low** N = 8[1]	**Normal** N = 26[1]	**p-value**[2]
Follicular destruction					0.2
Absent	6 (16%)	1 (33%)	0 (0%)	5 (19%)	
Present	31 (84%)	2 (67%)	8 (100%)	21 (81%)	
Vacuolation in epithelial cells					>0.9
Absent	5 (14%)	0 (0%)	1 (13%)	4 (15%)	
Present	32 (86%)	3 (100%)	7 (88%)	22 (85%)	
Crystallization in the follicle					>0.9
Absent	34 (92%)	3 (100%)	7 (88%)	24 (92%)	
Present	3 (8.1%)	0 (0%)	1 (13%)	2 (7.7%)	
Engorgement of stromal capillaries.					0.3
Absent	3 (8.1%)	1 (33%)	0 (0%)	2 (7.7%)	
Present	34 (92%)	2 (67%)	8 (100%)	24 (92%)	
Parenchymatous hemorrhage					0.4
Absent	26 (70%)	1 (33%)	7 (88%)	18 (69%)	
Present	11 (30%)	2 (67%)	1 (13%)	8 (31%)	
Intrafollicular aggregates of RBC’s					0.6
Absent	31 (84%)	2 (67%)	7 (88%)	22 (85%)	
Present	6 (16%)	1 (33%)	1 (13%)	4 (15%)	
Variation in follicular size					>0.9
Absent	30 (81%)	3 (100%)	7 (88%)	20 (77%)	
Present	7 (19%)	0 (0%)	1 (13%)	6 (23%)	

Characteristic	Overall N = 37[1]	High N = 3[1]	Low N = 8[1]	Normal N = 26[1]	p-value[2]
Metachromasia					0.6
Absent	22 (59%)	2 (67%)	6 (75%)	14 (54%)	
Present	15 (41%)	1 (33%)	2 (25%)	12 (46%)	
Inflammation					0.7
Absent	24 (65%)	2 (67%)	4 (50%)	18 (69%)	
Acute inflammation	1 (2.7%)	0 (0%)	0 (0%)	1 (3.8%)	
Chronic inflammation	12 (32%)	1 (33%)	4 (50%)	7 (27%)	
[1] n (%)					
[2] Fisher's exact test					

In this table, histopathological findings were based on TT4 levels for 37 cases.

Follicular Destruction:

- 6 cases (16%) showed no destruction, while 31 cases (84%) did.
- High TT4: 1 individual (33%) had no destruction, 2 individuals (67%) did.
- Low TT4: All 8 individuals (100%) had destruction.
- Normal TT4: 5 individuals (19%) had no destruction, 21 individuals (81%) did.
- p-value: 0.2, no significant difference.

Vacuolation in Epithelial Cells:

- 5 cases (14%) had no vacuolation, while 32 cases (86%) did.
- High TT4: All 3 individuals (100%) had vacuolation.
- Low TT4: 1 individual (13%) had no vacuolation, 7 individuals (88%) did.
- Normal TT4: 4 individuals (15%) had no vacuolation, 22 individuals (85%) did.
- p-value: >0.9, no significant difference.

Crystallization in the Follicle:

- 34 cases (92%) had no crystallization, while 3 cases (8.1%) did.
- High TT4: All 3 individuals (100%) had no crystallization.

- Low TT4: 1 individual (13%) had crystallization, 7 individuals (88%) did not.
- Normal TT4: 2 individuals (7.7%) had crystallization, 24 individuals (92%) did not.
- p-value: >0.9, no significant difference.

Engorgement of Stromal Capillaries:

- 3 cases (8.1%) had no engorgement, while 34 cases (92%) did.
- High TT4: 1 individual (33%) had no engorgement, 2 individuals (67%) did.
- Low TT4: All 8 individuals (100%) had engorgement.
- Normal TT4: 2 individuals (7.7%) had no engorgement, 24 individuals (92%) did.
- p-value: 0.3, no significant difference.

Parenchymatous Hemorrhage:

- High TT4: All 2 individuals (67%) had hemorrhage.
- Low TT4: 1 individual (13%) had hemorrhage, 7 individuals (88%) did not.
- Normal TT4: 18 individual (69%) had no hemorrhage, 8 individuals (31%) did.
- p-value: 0.4, no significant difference.

Intrafollicular Aggregates of RBC's:

- 6 cases (16%) had aggregates, while 31 cases (84%) did not.
- High TT4: 1 individual (33%) had aggregates, 2 individuals (67%) did not.
- Low TT4: 1 individual (13%) had aggregates, 7 individuals (88%) did not.
- Normal TT4: 4 individuals (15%) had aggregates, 22 individuals (85%) did not.
- p-value: 0.6, no significant difference.

Variation in Follicular Size:

- 7 cases (19%) had variation, while 30 cases (81%) did not.
- High TT4: All 3 individuals (100%) had no variation.
- Low TT4: 1 individual (13%) had variation, 7 individuals (88%) did not.
- Normal TT4: 6 individuals (23%) had variation, 20 individuals (77%) did not.
- p-value: >0.9, no significant difference.

Metachromasia:

- 15 cases (41%) had metachromasia, while 22 cases (59%) did not.
- High TT4: 1 individual (33%) had metachromasia, 2 individuals (67%) did not.

- Low TT4: 2 individuals (25%) had metachromasia, 6 individuals (75%) did not.
- Normal TT4: 12 individuals (46%) had metachromasia, 14 individuals (54%) did not.
- p-value: 0.6, no significant difference.

Inflammation:

- 12 cases (32%) had chronic inflammation, 1 case (2.7%) had acute inflammation, while 24 cases (65%) had no inflammation.
- High TT4: 1 individual (33%) had chronic inflammation, 2 individuals (67%) did not.
- Low TT4: 4 individuals (50%) had chronic inflammation, 4 individuals (50%) did not.
- Normal TT4: 7 individuals (27%) had chronic inflammation, 1 individual (3.8%) had acute inflammation, 18 individuals (69%) did not.

FT4

Table 44: FT4 in relation to histopathology

Characteristic	**Overall** N = 35[1]	**High** N = 2[1]	**Low** N = 2[1]	**Normal** N = 31[1]	**p-value**[2]
Follicular destruction					>0.9
Absent	6 (17%)	0 (0%)	0 (0%)	6 (19%)	
Present	29 (83%)	2 (100%)	2 (100%)	25 (81%)	
Vacuolation in epithelial cells					>0.9
Absent	5 (14%)	0 (0%)	0 (0%)	5 (16%)	
Present	30 (86%)	2 (100%)	2 (100%)	26 (84%)	
Crystallization in the follicle					>0.9
Absent	33 (94%)	2 (100%)	2 (100%)	29 (94%)	
Present	2 (5.7%)	0 (0%)	0 (0%)	2 (6.5%)	
Engorgement of stromal capillaries.					>0.9
Absent	3 (8.6%)	0 (0%)	0 (0%)	3 (9.7%)	
Present	32 (91%)	2 (100%)	2 (100%)	28 (90%)	
Parenchymatous hemorrhage					0.4
Absent	25 (71%)	1 (50%)	1 (50%)	23 (71%)	
Present	10 (29%)	1 (50%)	1 (50%)	8 (29%)	
Intrafollicular aggregates of RBC’s					0.13
Absent	29 (83%)	1 (50%)	1 (50%)	27 (87%)	
Present	6 (17%)	1 (50%)	1 (50%)	4 (13%)	
Variation in follicular size					0.5
Absent	29 (83%)	2 (100%)	1 (50%)	26 (84%)	
Present	6 (17%)	0 (0%)	1 (50%)	5 (16%)	

Characteristic	Overall N = 35[1]	High N = 2[1]	Low N = 2[1]	Normal N = 31[1]	p-value[2]
Metachromasia					0.8
Absent	22 (63%)	1 (50%)	2 (100%)	19 (61%)	
Present	13 (37%)	1 (50%)	0 (0%)	12 (39%)	
Inflammation					0.8
Absent	23 (66%)	2 (100%)	1 (50%)	20 (65%)	
Chronic inflammation	12 (34%)	0 (0%)	1 (50%)	11 (35%)	
[1] n (%)					
[2] Fisher's exact test					

In this table, histopathological findings were categorized for 35 cases based on FT4 levels.

Follicular Destruction:

- Overall: 6 cases (17%) showed no destruction, while 29 cases (83%) did.
- High FT4: All 2 individuals (100%) had destruction.
- Low FT4: All 2 individuals (100%) had destruction.
- Normal FT4: 6 individuals (19%) had no destruction, 25 individuals (81%) did.
- p-value: >0.9, no significant difference.

Vacuolation in Epithelial Cells:

- Overall: 5 cases (14%) had no vacuolation, while 30 cases (86%) did.
- High FT4: All 2 individuals (100%) had vacuolation.
- Low FT4: All 2 individuals (100%) had vacuolation.
- Normal FT4: 5 individuals (16%) had no vacuolation, 26 individuals (84%) did.
- p-value: >0.9, no significant difference.

Crystallization in the Follicle:

- Overall: 33 cases (94%) had no crystallization, while 2 cases (5.7%) did.
- High FT4: Both individuals (100%) had no crystallization.
- Low FT4: Both individuals (100%) had no crystallization.
- Normal FT4: 2 individuals (6.5%) had crystallization, 29 individuals (94%) did not.

- p-value: >0.9, no significant difference.

Engorgement of Stromal Capillaries:

- Overall: 3 cases (8.6%) had no engorgement, while 32 cases (91%) did.
- High FT4: Both individuals (100%) had engorgement.
- Low FT4: Both individuals (100%) had engorgement.
- Normal FT4: 3 individuals (9.7%) had no engorgement, 28 individuals (90%) did.
- p-value: >0.9, no significant difference.

Parenchymatous Hemorrhage:

- High FT4: 1 individual (50%) had hemorrhage, 1 individual (50%) did not.
- Low FT4: 1 individual (50%) had hemorrhage, 1 individual (50%) did not.
- Normal FT4: 23 individual (71%) had no hemorrhage, 8 individuals (29%) did.
- p-value: 0.4, no significant difference.

Intrafollicular Aggregates of RBC's:

- Overall: 29 cases (83%) had no aggregates, while 6 cases (17%) did.
- High FT4: 1 individual (50%) had aggregates, 1 individual (50%) did not.
- Low FT4: 1 individual (50%) had aggregates, 1 individual (50%) did not.
- Normal FT4: 4 individuals (13%) had aggregates, 27 individuals (87%) did not.
- p-value: 0.13, no significant difference.

Variation in Follicular Size:

- Overall: 29 cases (83%) had no variation, while 6 cases (17%) did.
- High FT4: Both individuals (100%) had no variation.
- Low FT4: 1 individual (50%) had no variation, 1 individual (50%) did.
- Normal FT4: 5 individuals (16%) had variation, 26 individuals (84%) did not.
- p-value: 0.5, no significant difference.

Metachromasia:

- Overall: 22 cases (63%) had no metachromasia, while 13 cases (37%) did.
- High FT4: 1 individual (50%) had metachromasia, 1 individual (50%) did not.
- Low FT4: 2 individuals (100%) had no metachromasia.
- Normal FT4: 12 individuals (39%) had metachromasia, 19 individuals (61%) did not.

- p-value: 0.8, no significant difference.

Inflammation:

- Overall: 23 cases (66%) had no inflammation, 12 cases (34%) had chronic inflammation.
- High FT4: 2 individuals (100%) had no inflammation.
- Low FT4: 1 individual (50%) had no inflammation, 1 individual (50%) had chronic inflammation.
- Normal FT4: 20 individuals (65%) had no inflammation, 11 individuals (35%) had chronic inflammation.
- p-value: 0.8, no significant difference.

CHAPTER 6: DISCUSSION

DISCUSSION

Hanging is a common and lethal method of suicide worldwide, with a fatality rate over 70%(7). It is particularly prevalent among young people, especially males(65). Factors contributing to hanging suicides include psychiatric conditions, social upheaval, and cultural influences(66,67). While most cases are suicides, accidental deaths and rare homicides have been reported(68,69). Most hanging suicides occur in the community, with only about 10% in controlled environments like prisons or hospitals(7).

The challenge of gathering evidence in hanging cases has long troubled medicolegal experts. This difficulty often arises because such incidents typically happen in secluded or hidden locations, complicating the collection of reliable forensic data. In these scenarios, the absence of witnesses or clear-cut physical evidence can make it difficult to determine the precise circumstances surrounding the death. This ambiguity not only hampers investigations but also poses significant hurdles in establishing the intent, whether it was a result of foul play, accidental, or a deliberate act. Homicidal hangings can be challenging to distinguish from suicides, often requiring detailed scene investigation and autopsy findings(70). The current study focused on 37 hanging cases and 39 control cases. It included all qualifying hanging cases brought for autopsy to the Department of Forensic Medicine at AIIMS Bhopal. The goal was to deeply investigate the involvement of the thyroid gland in these cases, given its anatomical position, and to determine any potential connections. While this methodology has been employed in various international studies, it is being applied for the first time in India.

AGE DISTRIBUTION

We found that the majority of hanging cases in the study were in between the ages of 20 and 40 years. The mean age was 30 years with a standard deviation of 10 years. Age distribution showed that 8 (22%) individuals were below 20 years, 22 (59%) were between 20 and 40 years, and 7 (19%) were above 40 years.

The higher hanging rates in the 20-40 age group can be attributed to several factors. This age range often encompasses individuals facing significant life stressors, such as career pressures, financial responsibilities, relationship issues, and mental health challenges. The table below shows the comparison studies.

GENDER DISTRIBUTION

Of the cases, 13 (35%) were female and 24 (65%) were male, showing a notable prevalence in males. In the current investigation, the male to female ratio was 1.85:1. The high incidence of hanging among men could be linked to the continuous stress of being the primary family provider. This burden, often tied to societal expectations based on gender, places significant pressure on men. However, the main reason for their higher representation in these cases is that men constitute the majority of the autopsy population.

MARITAL STATUS

Regarding marital status, 48 (63%) of the individuals were married. The increased incidence of hanging in the married population can be attributed to several factors. Married individuals often face significant stressors related to family responsibilities, financial pressures, and interpersonal conflicts. Additionally, the presence of marital disputes and domestic violence can further exacerbate these feelings, increasing the risk of suicide.

EDUCATIONAL STATUS

As per our study, out of a total of 37 hanging cases, 11 individuals (30%) had primary or lower education, while the majority, 26 (70%), had higher educational levels. Specifically, 14 individuals (38%) had attended high school, 9 cases (24%) had higher secondary education, and 3 cases (8.1%) had pursued higher studies. This trend may be due to rising literacy rates in the nation and increased public awareness of the importance of education.

Higher suicide rates among literate individuals may be attributed to increased stress from financial and employment pressures, debt, and the competitive nature of education.

RELIGION

As per our study, out of a total of 76 cases, 72 individuals (95%) were Hindu, while 4 individuals (5.3%) were non-Hindu. This aligns with the predominantly Hindu population in the region. Apart from the demographic predominance, the increase in Hindu cases could be linked to the cultural and societal pressures specific to this community. Factors such as stringent social expectations, family honour, and stigmas associated with mental health can contribute to higher stress levels. Additionally, traditional practices and beliefs might sometimes impede individuals from seeking help, leading to a higher incidence within the Hindu population.

OCCUPATIONAL STATUS

Non-skilled workers, who form the largest group (49%), often face unstable job conditions, low income, and harsh working environments, all of which can contribute to significant stress and mental health issues. Students, accounting for 24% of the cases, are under constant pressure to perform academically and secure their future, which can lead to heightened anxiety and despair. Skilled workers, comprising 16% of the cases, although more stable than non-skilled workers, still encounter job-related pressures and the need to maintain a certain standard of living. Housewives, making up 11% of the cases, may experience isolation, lack of personal fulfilment, and overwhelming family responsibilities and disputes which contribute to the hanging.

PERSONAL AND PAST HISTORY

In our study, regarding substance use a considerable number of individuals consumed alcohol (41%) and used chewing tobacco (41%), with 24% being smokers and 8.1% using drugs. Substance use often serves as a coping mechanism for stress and anxiety, but it can worsen mental health issues, impair judgment, and lower inhibitions, potentially leading to suicidal behaviour. Additionally, 8.1% had hypertension, 5.4% had undergone surgical treatment, 8.1% had a history of mental illness, and 5.4% had previous medical treatment. Diabetes was present in 2.7% of the cases. These pre-existing conditions likely contributed to the stress and vulnerability of the individuals. It's also important to note that improper or incomplete history may have further undermined these findings.

GENERAL AUTOPSY FINDINGS

In our current study, the significant difference in facial congestion between hanging cases (95%) and the control group (5.4%) and similarly in case of cyanosis in hanging cases (78%) compared to the control group (33were seen. However, petechiae was distributed in both groups, did not show a significant difference suggesting that petechiae might not be as reliable an indicator for distinguishing between hanging and control cases.

CHARACTERISTICS OF HANGING

In our study, the predominant use of synthetic ligature material (51%) can be attributed to its availability and strength. Cotton (31%), nylon (11%), and jute (5.7%) were also used, reflecting varying access and choices. Soft ligature materials were preferred (69%) due to their flexibility and ease of application. Most cases (89%) were complete hangings, likely because they ensure the outcome intended by the individual. Prominent and grooved ligature mark impressions (92%) were majority, as the force applied in hanging often leaves clear marks with time suspended also playing a part. The majority of knots were positioned posteriorly (73%) with lateral in 9 (24%) cases, and anterior in 1 (2.7%) case.

GROSS APPEARANCE OF THYROID GLAND

In our study of the 37 hanging cases, 23 (62%) showed a congested thyroid, while 14 (38%) appeared normal. This congestion occurs as the blood flow is restricted during hanging, causing pooling of blood. Conversely, the normal appearance of the thyroid in 14 cases (38%) could be due to variations in the duration of suspension and the individual's physical condition at the time of death, which might influence the degree of vascular congestion. The mean thyroid weight in females is 11.61 grams (range: 8 to 16 grams), while in males it is higher at 15.58 grams (range: 8 to 21 grams). These trends in weight may be due to males have larger body sizes and greater organ and body mass.

HISTOPATHOLOGY OF THYROID IN CASES OF HANGING

In our research, we found follicular destruction in 84% of hanging cases. It was more common in deaths that had occurred more recently, indicating it's not dependent on the time since death and likely not a result of postmortem changes. This implies that the breakdown of follicular structures happens immediately following death and doesn't continue significantly post-mortem. This is similar in cases described by Ikeda T et al where 62.5% cases showed follicular destruction.(59)

Vacuolation in epithelial cells was present in 86% of cases, showing slight increase over time. Vacuolation, the formation of vacuoles within cells, likely occurs as cells begin to degenerate and lose structural integrity, influenced most probably by factors such as autolysis which can

vary based on environmental conditions and the elapsed time post-mortem. This is different in cases described by Ikeda T et al where 37.5% cases showed vacuolation.(59)

Crystallization in the follicle was rare, present in only 8.1% of cases, showing slight increase over time. The low prevalence of crystallization indicates it's an uncommon post-mortem change, possibly due to dehydration which occurs with time. This is different in cases described by Ikeda T et al where 25% cases showed crystallisation.(59)

Engorgement of stromal capillaries was highly prevalent, seen in 92% of cases, and consistently observed across all time groups. This suggests that capillary engorgement is an early and persistent post-mortem change, likely due to congestion commonly seen in hanging deaths. This is similar in cases described by Magdalena Okłota et al(100%) and Pathak Harish et al(87.8%)showed segmental blood vessel expansion and congestion respectively.(57,58)

Parenchymatous hemorrhage was present in 30% of cases, with its prevalence showing a slight to no increase over time. Haemorrhages occurs as blood vessels break down, allowing blood to leak into surrounding tissues. The finding suggests traumatic destruction of vessel walls causing extravasation at time of death. This is similar in cases described by Magdalena Okłota et al(33%) and different in cases described by Ikeda T et al(0%) where no cases showed parenchymatous or petechial hemorrhage of thyroid gland.(57,59)

Metachromasia, seen in 41% of cases, remained consistent across time groups. This change in tissue staining properties can be associated with the breakdown of cellular components and changes in the chemical composition of tissues as decomposition progresses which can vary based on environmental conditions.

Intrafollicular RBC aggregates were noted in 16% of cases, with no significant variations among different time groups. Suggesting minimal extravasation into intact follicles.

Variation in follicular size was observed in 19% of cases. This is similar in cases described by Ikeda T et al (16.7%). (59) Indicating that follicular structures having varied sizes in a lower percentage of population and does not significantly associate with the cause of death.

Inflammation was present in 35% of cases, with chronic inflammation being more common (32%) than acute inflammation (2.7%). The presence of chronic inflammation suggests ongoing tissue responses to pre-mortem injuries or infections suggestive of prevalence of non-diagnosed thyroid or systemic inflammatory diseases.

Table 45: Histopathology of thyroid in various studies

S.N	Authors	Year	Sample population	Sample size	Findings
1	Magdalena Okłota et al(57)	2011	Vertical hanging cases	NA	Petechiae were observed in 33% of cases. All cases showed thyroid hyperaemia and segmental blood vessel expansion.
2	Pathak Harish et al(58)	2022	Hanging deaths.	41	Congestion in 36 cases (87.8%) and focal interstitial infiltration in five cases (12.2%)
3	Ikeda T et al(59)	2017	Hanging	8 in 96 samples	Follicular destruction:62.5% Vacuolation:37.5% Crystallization in the follicle:25% Parenchymatous hemorrhage:0% Follicular size variation:16.7% Follicular metachromasy:16.7%
4	Ikeda T et al(59)	2017	systemic ischemia/hypoxia	96	No differences were observed with respect to the cause of death in terms of follicular vacuolation, follicular crystallization, parenchymatous hemorrhage, follicular size, or follicular metachromasia.

BIOCHEMICAL MARKERS

In our study, we analysed 76 cases and found significant differences in thyroid function tests between the cases and control groups. The T3 and TT4 levels showed increased concentrations and was significant in hanging cases when compared to the controls. TT4 and T3 showed cutoff values of 6.88 µg/dl and 0.96 ng/ml for hanging cases with relatively high specificity and sensitivity. The observed rise in T3 and T4 levels aligns with the findings of N. Tani et al. (2020) and Muller et al. (1991)(38,41). This suggests that TT4 and T3 levels can serve as definitive indicators of hanging in cases where other findings are inconclusive.

Thyroglobulin levels generally increased with cases showing a mean of 131 ng/ml (SD 137) and controls at 191 ng/ml (SD 147), suggesting a significant variance($p = 0.044$) in thyroglobulin levels between the groups. While TSH and FT4 showed higher mean values in control group and

was not statistically significant. Thyroglobulin levels in predicting hanging showed a cutoff value of 125.77 ng/ml with specificity is 63.2% and the sensitivity is 63.9%.

The findings are differing to Rachut et al. (1980)(36) reported irregular T3 patterns and reduced T4 levels, Senol et al. (2008) identified elevated FT3 and Tg levels in hanging cases, Palmiere et al. (2018) noted an increase in Tg, T3, and FT3 in hanging cases, with T3 and FT3 levels increasing over time, while TT4 and FT4 levels decreased, Marin Takaso et al. (2018) and Keiji Tamaki et al. (1990) established a Tg cutoff value of 200 ng/mL in neck compression cases(8,36,38,41,46,47). The mean Thyroglobulin (Tg) values in our study were lower than in other studies because we collected blood from peripheral sites. This suggests that peripheral blood Tg levels are not a definitive indicator of hanging or vital reactions.

Table 46:Biochemical markers of thyroid in various studies

S.N	Authors	Year/region	Sample population	Sample size	Findings
1	E. Rachut et al(36)	1980	All autopsy cases	NA	T3- irregular and variable patterns T4- decreased
2	Wellby et al(37)	1987	Sudden infant death syndrome	36	T3- increased T4 and fT4 - decreased
3	N. Tani et al(38)	2020	hypoxia/ischemia	96	T3, T4 increased in in cases of mechanical asphyxia and acute/subacute blunt head injury, indicating hypoxic and ischemic brain conditions.
4	Muller et al.(41)	1991	All autopsy cases	NA	T4- increased in hanging T3 - increased in both hanging and sudden natural deaths
5	Senol et al(8)	2008	25- hanging 25-sudden death	50	FT3 and Tg- increased in hanging
6	Palmiere et al(45)	2018	All autopsy cases	NA	Tg, T3 , FT3 increased in hanging T3, ft3 increased with time TT4, FT4 decreased

7	Marin Takaso et al.(46)	2018	All autopsy cases	256	No significant difference Tg levels neck compression cases and others. Frequency of neck compression was significantly higher in Tg levels ≥200 ng/mL. Tg levels in the right heart blood ~ 10x peripheral blood levels High Tg levels in ~ 50% of non-neck compression cases.
8	Keiji Tamaki et al(47)	1990	Neck compression vs Non neck compression	42 vs 36	Tg levels mostly above 200 ng/ml in neck compression cases.
9	Y. Katsumata et al(48)	1984	Laboratory-based experimental study	NA	Tg levels ≥5 μg /mL detected even after 3months from blood stains.

BIOCHEMICAL MARKERS IN RELATION TO HISTOPATHOLOGY

Histopathological findings showed no significant differences between normal and abnormal thyroglobulin levels, except for Metachromasia. Metachromasia was more frequently found in the abnormal thyroglobulin group, possibly due to the passive diffusion of thyroglobulin. However, this difference was statistically insignificant. This may be due to the slow and localized dissemination of colloid, which does not reach the peripheral blood vessels effectively because death in hanging cases usually occurs rapidly.

Histopathological findings revealed no statistically significant differences across low, normal, and high T3 levels. However, there was a noticeable trend where engorgement of stromal capillaries and parenchymatous hemorrhage increased progressively from low to high T3 levels. This trend suggests possible leakage and the conversion of colloid into T3 in the blood. Histopathological findings revealed no statistically significant differences across low, normal, and high TT4 and FT4 levels.

The findings were consistent with those reported by N. Tani et al., Senol et al., and Ikeda T et al.(8,38,59)

Table 47: Correlation between histopathology and biochemical markers of thyroid in various studies

S.N	Authors	Year/region	Sample population	Sample size	Findings
1	N. Tani et al(38)	2020	hypoxia/ischemia	96	No significant relation between thyroid hormone levels and histopathology
2	Senol et al.(8)	2008	25- hanging 25-sudden death	50	No significant relation between thyroid hormone levels and histopathology
3	Ikeda T et al(59)	2017	hypoxia/ischemia	96 samples	No significant relation between thyroid hormone levels and histopathology in hanging cases

CHAPTER 7: CONCLUSION

CONCLUSION

After meticulously conducting the study with thorough analysis, observation, and evaluation, the following conclusions are drawn from the study:

- The majority of hanging cases were males and between the ages of 20 and 40 years. Higher rates were observed among married, literate populations and non-skilled workers. Notably, 41% of the individuals consumed alcohol, and 41% used chewing tobacco.

- General autopsy findings indicated significant differences in cyanosis and facial congestion in hanging cases. Synthetic ligature materials were predominantly used, with 92% of cases showing prominent and grooved ligature mark impressions.

- Histopathological examinations revealed the most common findings in order: engorgement of stromal capillaries, vacuolation in epithelial cells, and follicular destruction. Inflammation was present in 35% of cases, suggesting a prevalence of undiagnosed thyroid or systemic inflammatory diseases.

- The levels of T3 and TT4 were significantly higher in hanging cases, with cutoff values of 6.88 µg/dl for TT4 and 0.96 ng/ml for T3, showing relatively high specificity and sensitivity. This suggests that TT4 and T3 levels can serve as definitive indicators of hanging in cases where other findings are inconclusive.

- Thyroglobulin levels generally increased, with cases showing a mean of 131 ng/ml (SD 137) and controls at 191 ng/ml (SD 147), suggesting a significant variance ($p = 0.044$) between the groups. However, Tg levels are not definitive indicators of hanging or vital reactions.

- Metachromasia was more frequently found in the abnormal thyroglobulin group, possibly due to the passive diffusion of thyroglobulin.

- Overall, histopathological findings showed no statistically significant differences across low, normal, and high levels of T3, TT4, and FT4.

CHAPTER 8: LIMITATIONS

LIMITATIONS

Autopsy studies on the thyroid gland's histopathology and biomarker levels are exceedingly scarce. The available research is limited, predominantly to animal studies and a few analytical methods. Some limitations of the current study include

- The study excluded all deaths which was due to neck compression other than hanging.

- The absence of Indian research correlating gross and histopathological findings with biochemical markers of the thyroid indeed limits the contextual applicability of the results. This means that the findings might not fully reflect the specific conditions or variations present in the Indian population.

- Statistically significant correlation between T3 and TT4 and hanging, there exists an overlap between the range of values obtained in both hanging and non- hanging cases; this could be ascribed to the small sample size of the study.

- Being an autopsy study, it's susceptible to recall bias. The death of a loved one can distort the family's memories of the deceased, potentially skewing the information regarding the circumstances of the death. It's challenging to assess someone's information based on others' knowledge of that person.

- Autolysis of thyroid tissue, which is a natural process that occurs after death, can make it difficult to interpret histopathological findings. This process can mimic or obscure changes that occurred before death, complicating accurate diagnosis.

- The study might not fully consider factors like environmental exposures, medical history, and comorbidities, which could be affecting the study variables.

- Post-mortem changes, including decomposition, can distort tissue histology, necessitating careful consideration of the post-mortem interval.

CHAPTER 9: FUTURE SCOPE

FUTURE SCOPE

The histopathological and biomarker levels in hanging cases are sparsely studied. Homicidal cases are infrequently but certainly sometimes staged as suicides by hanging. Determining the manner of death can be challenging when evidence has been tampered with. This area requires more focused attention and research. The following new perspectives can be explored further as a result of this study:-

1. The current study can significantly aid in determining the suicidal nature of death. This will be immensely valuable in medico-legal investigations, helping to bring justice to the deceased and their families.

2. Establishing TT4 and T3 levels as reliable markers for hanging can enhance the accuracy of forensic investigations, particularly in cases where other evidence is inconclusive.

3. The findings can be used to educate and train forensic pathologists and medical examiners, enabling them to recognize key histopathological changes and biochemical markers associated with hanging.

4. Data-driven insights from the study can assist policymakers in creating standardized forensic practices, leading to more reliable autopsy procedures globally.

5. The study paves the way for further research into the biochemical and histopathological aspects of hanging, encouraging more detailed investigations into various factors influencing post-mortem changes.

6. Understanding the prevalence of undiagnosed thyroid or systemic inflammatory diseases can have broader public health implications, potentially leading to better screening and preventive measures.

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CHAPTER 11: ANNEXURE

ANNEXURE

ANNEXURE I

Participant Information Sheet

1. **STUDY TITLE:** "Autopsy based study of histopathology and biochemical markers of thyroid gland in cases of hanging"
2. **STUDY INVESTIGATORS:**
   Student: Dr. Suhail Alikkal, Dept. of Forensic Medicine and Toxicology
   Guide: Dr. Atul S Keche, Dept. of Forensic Medicine and Toxicology
   Co- Guide: Dr. Arneet Arora, Dept. of Forensic Medicine and Toxicology
   Co- Guide: Dr. Ashwin Laxmikant Kotnis, Dept. of Biochemistry
   Co- Guide: Dr. Jai Kumar Chaurasia, Dept. of Pathology and Laboratory medicine.
3. **Introduction**:

We are conducting a research study to study histopathological and biochemical changes of thyroid gland in autopsy cases in AIIMS Bhopal. If you agree to take part voluntarily, we will ask you to sign a consent form.

4. **Purpose of the study:**

The aim of our study is to study changes related to thyroid gland in hanging and other cases. This will help us to understand the changes to thyroid gland and use this knowledge for medicolegal and medical treatment purposes

5. **Taking part – what it involves:**

a. Do I have to take part?

Participation in this research is entirely voluntary. A decision to not take part, or to withdraw at any time, will not affect your rights or medical care in any way.

b. What is expected of me if I take part?

If you participate, for the research purpose we will collect samples of thyroid gland and blood. They are collected in such a way that aesthetic of the body is still maintained and there is no gross harm or disfiguration to the body of the deceased and the process will not hamper the duration of the autopsy.

. How long will my part in the study last?

Your participation in the study would last for a one-time visit.

d. Do I have to come for extra visits or undergo extra investigations for the study?

The blood sample collected will undergo ELISA test in the Department of Biochemistry.
No additional visits are required.

e. What are the possible benefits in taking part?

The study is not going to be directly to directly for the participants and no reports will be provided.

f. What are the possible disadvantages and risks of taking part?

There are no major foreseeable disadvantages or risks because of the study. There may be risk of loss of privacy and confidentiality of information collected even though all possible measures will be taken to ensure safe data storage and access.

g. What happens to the information collected from me?

All information collected will be kept strictly confidential and not shared with anyone. Results from the study will be reported as group data and will not identify you in any way.

h. What if I change my mind regarding participation in the study?

You are entitled to change your mind and withdraw at any time during the study without any disadvantage or penalty.

i. Whom do I contact for more information or if I have further questions or concerns?

If you want any information or have questions or concerns, you can contact:

Dr. Suhail Alikkal
Post graduate student,
Department of Forensic medicine and Research
(8075365941,
suhail.jr2022@aiimsbhopal.edu.in)

Dr. Shashank Purwar
Member Secretary,
Institute Ethics Committee – Student Toxicology AIIMS, Bhopal
(7773010099,
ihecpgr@aiimsbhopal.edu.in)

<u>प्रतिभागी सूचना पत्र</u>

1. **अध्ययन का शीर्षक:** फांसी के मामलों में थायरॉयड ग्रंथि के हिस्टोपैथोलॉजी और बायोकेमिकल मार्करों का ऑटोप्सी आधारित अध्ययन

2. **अध्ययन जांचकर्ता**

छात्र: डॉ. सुहैल आलीकल , फॉरेंसिक मेडिसिन एंड टॉक्सिकोलॉजी विभाग
मार्गदर्शक (गाइड): डॉ. अतुल एस केचे , फॉरेंसिक मेडिसिन एंड टॉक्सिकोलॉजी विभाग
सह मार्गदर्शक/ कों : डॉ अरनीत अरोड़ा , फॉरेंसिक मेडिसिन एंड टॉक्सिकोलॉजी विभाग
डॉ. अश्विन लक्ष्मीकांत कोटनिस, जैव रसायन विभाग
डॉ. जय कुमार चौरसिया, पैथोलॉजी एवं लेबोरेटरी मेडिसिन विभाग

3. **परिचय:**

हम एम्स, भोपाल में ऑटोप्सी मामलों में थायरॉयड ग्रंथि के हिस्टोपैथोलॉजिकल और जैव रासायनिक परिवर्तनों का अध्ययन करने के लिए एक शोध अध्ययन कर रहे हैं। यदि आप स्वेच्छा से भाग लेने के लिए सहमत है तो, हम आपसे यह सहमति प्रपत्र हस्ताक्षर करने के लिए आग्रह करेंगे।

4. **अध्ययन का उद्देश्य:**

हमारे अध्ययन का उद्देश्य हैंगओवर और अन्य मामलों में थायरॉयड ग्रंथि से संबंधित परिवर्तनों का अध्ययन करना है। यह हमें थायरॉयड ग्रंथि में होने वाले परिवर्तनों को समझने में मदद करेगा और इस ज्ञान का औषधीय और चिकित्सा उपचार उद्देश्यों के लिए उपयोग करेगा

5. **भाग लेना - इसमें क्या शामिल है ?**

a. **क्या मुझे भाग लेना चाहिए ?**

इस अनुसंधान में भाग लेना पूर्णतः स्वैच्छिक है । भाग नहीं लेने का निर्णय या किसी भी समय अपना नाम वापस लेने से, किसी भी रूप में आपके अधिकारों या आपकी चिकित्सा देखभाल पर कोई विपरीत प्रभाव नहीं पड़ेगा ।

b. **यदि मैं इसमें शामिल होता हूं तो मुझसे क्या अपेक्षित हैं ?**

यदि आप भाग लेते हैं, तो शोध के उद्देश्य से हम थायरॉयड ग्रंथि और रक्त के नमूने एकत्र करेंगे। उन्हें इस तरह से एकत्र किया जाता है कि शरीर का सौंदर्य अभी भी बना रहता है और मृतक के शरीर को कोई गंभीर नुकसान या विरूपण नहीं होता है और यह प्रक्रिया शव परीक्षण की अवधि में बाधा नहीं बनेगी।

c. **अध्ययन में मेरी भागीदारी कब तक चलेगी?**

अध्ययन में आपकी भागीदारी एक बार की यात्रा के लिए रहेगी।

d. **क्या मुझे इस अध्ययन के लिए अलग से आना पड़ेगा या अतिरिक्त जांच जरूरी होगी ।**

एकत्र किए गए रक्त के नमूने को जैव रसायन विभाग में एलिसा परीक्षण से गुजरना होगा।
किसी अतिरिक्त विज़िट की आवश्यकता नहीं है।

e. **इसमें शामिल होने से संभावित लाभ क्या है ?**

अध्ययन सीधे प्रतिभागियों के लिए नहीं होगा और कोई रिपोर्ट प्रदान नहीं की जाएगी।

f. **इसमें भाग लेने से संभावित हानि एवं जोखिम ।**

अध्ययन की वजह से कोई बड़ा अनुमानित नुकसान या जोखिम नहीं हैं। एकत्र की गई जानकारी की गोपनीयता और गोपनीयता के नुकसान का जोखिम हो सकता है, भले ही सुरक्षित डेटा भंडारण और पहुंच सुनिश्चित करने के लिए सभी संभव उपाय किए जाएंगे।

g. **मेरे बारे में एकत्र की गई सूचना का क्या किया जाएगा ?**

एकत्र की गई सभी सूचना पूर्णतः गोपनीय रखी जाएगी तथा अध्ययन से प्राप्त निष्कर्षों को अन्य किसी से साझा नहीं किया जाएगा । इन्हें ग्रुप आंकड़े के रूप मे रिपोर्ट किया जाएगा और किसी भी तरह से आपकी पहचान उजागर नहीं होगी ।

h. **इस अध्ययन मे भाग लेने के लिए मेरे विचार बदलने पर क्या होगा ?**

अध्ययन के दौरान किसी भी समय बिना किसी क्षति या दंड के आपको अपने विचार बदलने का पूरा अधिकार है ।

I. **इस संबंध मे अधिक जानकारी के लिए या बाद मे मेरे मन मे कोई प्रश्न या शंका होने पर मुझ किनसे संपर्क करना होगा ?**

यदि आप कोई सूचना चाहते हैं या आपका कोई प्रश्न है या शंका है तो आप इन नंबरों पर संपर्क कर सकते हैं:

डॉ. सुहैल आलीकल डॉ. शशांक पुरवार
स्नातक छात्र, सदस्य सचिव,
फॉरेंसिक मेडिसिन एंड टॉक्सिकोलॉजी विभाग, संस्थान आचार समिति - छात्र अनुसंधान
एम्स, भोपाल
(8075365941, suhail.jr2022@aiimsbhopal.edu.in) (7773010099, ihecpgr@aiimsbhopal.edu.in)

ANNEXURE II

प्रतिभागी सूचना सहमति प्रपत्र

[PARTICIPANT INFORMED CONSENT FORM (PICF)]

इस अध्ययन के लिए प्रतिभागी का पहचान नंबरः …….....

Participant Identification Number for this Study: ……...

अनुसंधान का शीर्षकः फांसी के मामलों में थायरॉयड ग्रंथि के हिस्टोपैथोलॉजी और बायोकेमिकल मार्करों का ऑटोप्सी आधारित अध्ययन

Title of Project : Autopsy based study of histopathology and biochemical markers of thyroid gland in cases of hanging

शोधकर्ता छात्र का नामः डॉ. सुहैल आलीकल फोन नं: 8075365941.

Name of Student Investigator Dr. Suhail Alikkal Tel No.(s) : 8075365941

शोध मार्गदर्शक का नामः डॉ. अतुल एस केचे

Name of Thesis Guide : Dr. Atul S Keche

मैंने दिनांक 20.08.2022 के सूचना पत्रक संस्करण II में दिए गए सभी तथ्यों को सावधानीपूर्वक पढ़ लिया है/ मुझे अपनी भाषा में स्पष्ट रूप से विस्तारपूर्वक समझा दिया गया है, और मैंने तथ्यों को पूर्णतः समझ लिया है। मैं पुष्टि करता/ करती हूँ, कि मुझे सवाल पूछने के अवसर प्रदान किए गए। The contents of the information sheet version II dated 20 August 2022 that was provided have been read carefully by me / explained in detail to me, in a language that I comprehend, and I have fully understood the contents. I confirm that I have had the opportunity to ask questions.

मुझे इस अध्ययन की प्रकृति एवं उद्देश्य और इसके संभावित जोखिमों / लाभों तथा अध्ययन की अनुमानित अवधि एवं अन्य प्रासंगिक जानकारी के बारे में विस्तारपूर्वक समझा दिया गया है। मुझे पता है कि इस अध्ययन में मेरी भागीदारी स्वैच्छिक है तथा मैं इस अध्ययन से किसी भी समय बिना कोई कारण बताए, अपना नाम वापस लेने के लिए स्वतंत्र हूं और नाम वापस लेने पर मेरी चिकित्सा देखभाल या कानूनी अधिकारों पर कोई प्रभाव नहीं पड़ेगा। The nature and purpose of the study and its potential risks/benefits and expected duration of the study, and other relevant details of the study have been explained to me in detail. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal right being affected.

मैं समझता/समझती हूं कि इस अनुसंधान में मेरी भागीदारी से मेरे बारे में एकत्र की गई जानकारी और मेरे चिकित्सीय दस्तावेजों के किसी भी भाग को एम्स के जिम्मेदार व्यक्तियों द्वारा ही देखा जाएगा। मैं इन व्यक्तियों को मेरे रिकॉर्ड प्राप्त करने की अनुमति प्रदान करता/ करती हूँ। I understand that the information collected about me from my participation in this research and sections of any of my medical notes may be looked at by responsible individuals from AIIMS. I give permission for these individuals to have access to my records.

यदि प्रतिभागी बच्चा है तो उसकी मौखिक या लिखित सहमति प्राप्त की गई है। If participants is child, his/her verbal of written assent has been obtained.

मैं उक्त अध्ययन में भाग लेने के लिए सहमत हूं/I agree to take part in the above study.

_____

(प्रतिभागी के हस्ताक्षर / बांए अंगूठे का निशान) (Signature/Left Thumb Impression)

दिनांक/Date……स्थान/Place …….....

प्रतिभागी का नाम/Name of the participant…….....

पिता/पति/पत्नी का नाम /Son/Daughter/Spouse of…….

डाक का पूरा पता /Complete Postal Address …….....

अगर अशिक्षित हों / If illiterate.

सूचना पत्र को प्रतिभागी को पढ़ा और समझाया गया है और प्रश्न पूछने का अवसर दिया गया है। मैं पुष्टि करता हूं कि व्यक्ति ने स्वतंत्र रूप से अपनी सहमति दी है ।

The information sheet has been read and explained to the participant and given the opportunity to ask questions. I confirm that the individual has given consent freely.

कानूनी रूप से स्वीकार्य प्रतिनिधि या निष्पक्ष गवाह के हस्ताक्षर

Signature of Legally acceptable representative or impartial witness.

दिनांक/Date

एलएआर या निष्पक्ष गवाह का नाम / Name of LAR or impartial witness .

पता / Address :-

प्रमाणित किया जाता है कि उक्त सहमति मेरी उपस्थिति में प्राप्त की गई ।

This is to certify that the above consent has been obtained in my presence.

_____

छात्र शोधकर्ता के हस्ताक्षर दिनांक……स्थान……

Signature of the student Investigator Date …… Place ……

ANNEXURE III

History Proforma

1	PM No	Study no:-
2	Date:	
3	Name of deceased	
4	Age:	
5	Sex-Male/Female/Others	
6	Religion:	
7	Education:-	
8	Occupation:-	
9	Habits- Smoking- Alcohol- Tobacco- Drugs- Others-	
10	Details of previous medical/surgical treatment:	
11	Co-Morbidities: • Hypertension: • Diabetes mellitus: • Thyroid disease: Hyperthyroidism/Hypothyroidism/Others • Other diseases:-	
12	History of mental illness:	
13	Last seen alive-	
14	Place of incidence-	
15	Death Declaration: Date	Time
16	Information given by	Name:
		Relation:
		Contact No:
		Signature:

Data entry Proforma

Autopsy findings	
1	**Study no**:-
2	**Date**:
Ligature	
3	Material : Type: Hard/Soft/Firm
4	**Type of Hanging:** Complete/Partial
External findings	
5	Facial congestion: Present/Absent
6	Cyanosis(Nail Bed): Present/Absent
7	Petechiae: Present/Absent
8	**Ligature mark**: Impression: Faint/Prominent/Grooved Knot Position:
9	Relation to lobes of thyroid: Closest to **Superior/Inferior/both** half of **Right/Left/Both** lobes. (Ligature mark)
Thyroid gland	
10	Gross Appearance:
11	Weight:
12	Time since death:
13	Cause of Death:

Biochemistry observations

1	**Study no**:-	
2	Thyroglobulin (Tg)	
3	FT3	
4	FT4	
5	Total T4	
6	TSH	

Histopathology Observation Sheet

1	**Study no:-**
2	**Date:**
	Histopathological Findings
4	Follicular destruction:
5	Vacuolation in epithelial cells :
6	Crystallization in the follicle:
7	Intrafollicular aggregates of RBC's and engorgement of stromal capillaries.
8	Parenchymatous hemorrhage:
9	Changes in colloid:
	Others: Acute inflammation Ischemic changes

MASTER CHART

SL no	Pm No	Cause of death	Time since death within_hrs	Case type	Age	Sex	Education	Religion	Occupation	Married	Smoking	Alcohol	Tobacco	Drugs	Previous Surgical treatment	Hypertension	Diabetes	Thyroid disease	History of mental illness	Previous medical treatment	Facial congestion	Cyanosis	Petechiae	Ligature Material	Ligature Material Type	Type of hanging	Ligature mark impression	Knot position	Gross appearance	Thyroid weight	T3 ng/ml	FT4 ng/dl	TSH uIU/ml	Thyroglobulin	Follicular destruction	Vacuolation in epithelial cells	Crystalloid in the follicle	Engorgement of stromal capillaries.	Parenchymatous hemorrhage	Intrafollicular aggregates of RBC's	Variation in follicular size	Metachromasia	Inflammation
1	332/23	Hanging	24	Case	35	Male	Primary or below	Hindu	Non-skilled	Y	Y	Y	Y	Y	N	N	N	N	N	N	+	+	+	Synthetic	Soft	Complete	Prominent	Posterior	Normal	17	1.1		7.7	16.8	1.68	+	+	+	+	-	-	-	+	Acute
2	348/23	Hanging	6	Case	18	Female	Secondary	Hindu	Student	N	N	N	N	N	N	N	N	N	Y	N	+	-	+	Synthetic	Soft	Complete	Prominent	Posterior	Congested	16		1.3	8.2	17.6	0.65	+	+	-	+	-	+	+	+	Chronic
3	367/23	Hanging	12	Case	29	Female	Secondary	Hindu	Student	Y	N	N	N	N	N	N	N	N	N	N	+	-	-	Cotton	Soft	Complete	Prominent	Posterior	Congested	11	1.3		7	23	3.73	+	+	-	+	+	+	+	+	.
4	366/23	Hanging	12	Case	52	Female	Primary or below	Hindu	Housewife	Y	N	N	N	N	N	Y	N	N	Y	N	+	+	-	Synthetic	Soft	Complete	Prominent	Posterior	Congested	12	1.4	1.3	9.8	18.5	7.74	+	+	-	+	+	-	+	-	.
5	383/23	Hanging	12	Case	17	Female	Secondary	Hindu	Student	N	N	N	N	N	N	N	N	N	N	N	+	+	-	Synthetic	Soft	Complete	Prominent	Posterior	Congested	12	2.6	1.2	9.9	7.55	1.66	+	+	+	+	+	-	+	-	.
6	388/23	Hanging	6	Case	30	Female	Secondary	Hindu	Skilled	Y	N	N	N	N	N	N	N	N	N	N	+	+	-	Synthetic	Soft	Complete	Prominent	Posterior	Congested	11	1	0.9	7.9	31.1	0.46	+	+	-	+	-	+	+	-	.
7	394/23	Hanging	12	Case	24	Male	Higher studies	Hindu	Student	N	N	N	N	N	N	N	N	N	N	N	+	+	+	Cotton	Hard	Complete	Prominent	Posterior	Congested	21	3.1	1.4	9.6	54.3	1.4	+	+	-	+	+	-	-	-	.
8	398/23	Hanging	12	Case	35	Male	Highschool	Non-Hindu	Non-skilled	Y	N	N	N	N	N	N	N	N	N	N	+	+	-	Cotton	Hard	Complete	Prominent	Posterior	Normal	16	1.7	1	8.3	434	4.84	+	+	-	+	+	-	-	-	.
9	422/23	Hanging	12	Case	42	Male	Highschool	Hindu	Non-skilled	Y	N	Y	Y	N	N	N	N	N	N	N	+	+	-	Synthetic	Soft	Complete	Prominent	Posterior	Normal	19	7.8	1.4	11	109	2.65	+	+	-	+	-	-	+	+	Chronic
10	439/23	Hanging	12	Case	19	Male	Primary or below	Hindu	Non-skilled	N	N	N	N	N	N	N	N	N	N	N	+	+	+	Cotton	Soft	Complete	Faint	Anterior	Congested	14	2.4	1.2	8.4	98.7	1.14	+	+	-	+	+	+	-	-	.
11	468/23	Hanging	24	Case	20	Male	Secondary	Hindu	Student	N	Y	Y	Y	N	N	N	N	N	N	N	+	+	-	Jute	Hard	Complete	Grooved	Posterior	Normal	15	2.9	1.6	13	362	3.6	-	+	-	+	+	+	-	-	Chronic
12	509/23	Hanging	12	Case	26	Male	Highschool	Hindu	Non-skilled	Y	N	Y	Y	N	N	N	N	N	N	N	+	+	-	Synthetic	Soft	Partial	Prominent	Lateral	Normal	14	1.8	1	6.9	69.7	5.09	+	+	-	+	-	-	-	-	Chronic
13	534/23	Hanging	6	Case	26	Female	Higher studies	Hindu	Skilled	Y	N	N	N	N	N	N	N	N	N	N	+	-	-	Nylon	Hard	Complete	Prominent	Posterior	Congested	8	1.1	0.8	4.3	4.17	7.55	+	+	-	+	-	+	-	-	Chronic
14	552/23	Hanging	24	Case	24	Female	Highschool	Hindu	Non-skilled	Y	N	N	Y	N	N	N	N	Y	N	N	+	+	-	Cotton	Soft	Complete	Prominent	Lateral	Congested	9	5.6	1.1	8.3	190	1.72	-	-	-	+	+	+	-	-	.
15	553/23	Hanging	24	Case	20	Male	Secondary	Hindu	Student	N	Y	N	N	N	N	N	N	N	N	N	-	+	+	Cotton	Soft	Partial	Prominent	Lateral	Normal	13		1.4	12	51.2	0.8	-	+	-	+	-	-	-	-	.
16	567/23	Hanging	24	Case	22	Male	Highschool	Hindu	Non-skilled	N	Y	Y	Y	N	N	N	N	N	N	N	+	-	-	Cotton	Soft	Complete	Prominent	Posterior	Congested	17	2.2	1.5	10	404	1.52	+	+	-	+	-	-	-	-	.
17	601/23	Hanging	12	Case	35	Female	Primary or below	Hindu	Housewife	Y	N	Y	Y	N	N	N	N	N	N	Y	+	+	-	Synthetic	Soft	Complete	Faint	Posterior	Normal	11	1.2	1.5	9.4	14.7	0.72	-	+	-	+	-	-	-	-	Chronic
18	604/23	Hanging	6	Case	52	Male	Primary or below	Hindu	Non-skilled	Y	Y	Y	Y	N	N	Y	Y	N	N	N	+	-	-	Synthetic	Soft	Partial	Prominent	Lateral	Normal	18	1.3	1.6	12	45.9	1.22	+	+	-	-	+	-	-	-	.
19	611/23	Hanging	6	Case	30	Male	Secondary	Hindu	Student	Y	Y	N	N	N	N	N	N	N	N	N	+	+	-	Jute	Hard	Complete	Grooved	Posterior	Normal	14		1.9	10	124	0.71	+	+	-	+	+	+	-	+	.
20	612/23	Hanging	24	Case	25	Male	Highschool	Hindu	Non-skilled	N	N	Y	Y	N	N	N	N	N	N	N	+	+	-	Synthetic	Soft	Complete	Grooved	Posterior	Normal	15	6.8	1.4	9.1	223	1.74	+	+	-	+	-	-	-	+	Chronic
21	617/23	Hanging	24	Case	25	Male	Highschool	Hindu	Non-skilled	N	N	Y	N	N	N	N	N	N	N	N	+	+	-	Synthetic	Soft	Complete	Prominent	Posterior	Normal	21	1.4	1	6	3.68	1	+	+	+	+	-	-	-	-	Chronic
22	623/23	Hanging	12	Case	31	Female	Highschool	Hindu	Housewife	Y	N	N	Y	N	N	N	N	N	N	N	+	-	-	Nylon	Hard	Complete	Grooved	Posterior	Normal	10	4.3	1.3	8.7	126	0.91	+	+	-	+	-	-	-	+	.
23	055/24	Hanging	12	Case	31	Male	Primary or below	Hindu	Non-skilled	Y	N	Y	Y	N	Y	N	N	N	N	N	-	-	+	Synthetic	Soft	Complete	Grooved	Posterior	Congested	15	1.5	1.2	7.1	0.83	3.52	-	-	-	-	-	-	-	+	.
24	060/24	Multiple injuries	12	Control	35	Male	Primary or below	Hindu	Non-skilled	Y	N	N	Y	N	N	N	N	N	N	N	-	-	+						Normal	17	0.9	1.3	6.6	462	0.55									
25	094/24	Hanging	24	Case	18	Male	Highschool	Hindu	Non-skilled	N	N	N	N	N	N	N	N	N	N	N	+	+	+	Synthetic	Soft	Complete	Grooved	Posterior	Congested	16	1.2	1	7.4	6.77	0.341	+	+	-	+	-	-	-	-	Chronic
26	104/24	Hanging	6	Case	41	Male	Primary or below	Hindu	Non-skilled	Y	N	Y	N	N	N	N	N	N	N	N	+	+	-	Nylon	Hard	Complete	Prominent	Lateral	Congested	15	1.1	0.9	4.9	6.2	0.776	+	-	-	+	-	-	-	+	.
27	130/24	Hanging	24	Case	26	Male	Primary or below	Hindu	Non-skilled	Y	N	N	N	N	N	N	N	N	N	N	+	+	-	Cotton	Soft	Complete	Faint	Posterior	Congested	16	1.2	1	11	332	3.733	+	+	-	+	-	-	-	-	.
28	150/24	Hanging	24	Case	26	Male	Highschool	Hindu	Non-skilled	N	Y	Y	N	N	N	N	N	N	N	N	+	+	+	Cotton	Hard	Complete	Grooved	Lateral	Normal	18	1.2	1	6.6	296	1.439	+	+	-	+	-	-	-	+	.
29	204/24	Hanging	12	Case	38	Male	Secondary	Hindu	Skilled	Y	N	N	N	N	N	N	N	N	N	N	+	+	-	Synthetic	Soft	Complete	Prominent	Posterior	Congested	16	1.1	1	6.8	202	0.938	-	+	-	+	-	-	-	+	.
30	205/24	Hanging	12	Case	49	Female	Highschool	Hindu	Housewife	Y	N	N	N	N	N	N	N	N	N	N	+	-	-			Complete	Grooved	Posterior	Congested	10	1	1	5.2	264	1.589	+	+	-	+	-	-	-	+	Chronic
31	212/24	Multiple injuries	12	Control	24	Male	Secondary	Hindu	Student	N	Y	N	N	N	N	N	N	N	N	N	-	-	-						Normal	18	1.1	1.1	6.1	461	1.643									
32	218/24	Hanging	12	Case	45	Male	Secondary	Hindu	Skilled	Y	Y	Y	Y	N	N	Y	N	N	N	N	+	+	-	Nylon	Hard	Complete	Grooved	Lateral	Congested	13	2	1.2	21	437	2.918	+	+	-	+	-	-	-	+	.
33	228/24	Hanging	12	Case	30	Male	Primary or below	Hindu	Non-skilled	Y	N	Y	Y	N	N	N	N	N	N	N	+	+	-	Synthetic	Hard	Complete	Grooved	Lateral	Congested	17	1	0.8	4.8	88.6	1.907	+	+	-	+	+	-	+	-	.
34	229/24	Cardiac Pathology	6	Control	16	Female	Highschool	Hindu	Student	N	N	N	N	N	N	N	N	N	N	N	-	+	-						Pale	14	0.5	0.6	3.4	48	0.786									
35	233/24	Head Injury	12	Control	35	Male	Highschool	Hindu	Non-skilled	Y	N	N	N	N	N	N	N	N	N	N	-	-	-						Normal	15	0.9	1.3	6.6	79.6	16.47									
36	242/24	Lung pathology	6	Control	27	Male	Highschool	Hindu	Skilled	N	Y	Y	N	N	N	N	N	N	N	N	-	+	-						Normal	12	1	1.2	8.1	3.54	2.106									
37	253/24	Poisoning	6	Control	16	Female	Highschool	Hindu	Student	N	N	N	N	N	N	N	N	N	N	N	-	-	-						Normal		1.6	0.9	4.8	298	2.217									
38	254/24	Hanging	12	Case	14	Male	Highschool	Hindu	Student	N	N	N	N	N	N	N	N	N	N	N	+	+	+	Synthetic	Soft	Complete	Prominent	Posterior	Congested	12	2.8	2.4	5.8	25.3	0.68	+	+	-	+	-	-	-	-	.
39	285/24	Hanging	12	Case	39	Male	Primary or below	Hindu	Non-skilled	Y	N	Y	Y	N	N	N	N	N	N	N	+	+	-			Complete	Prominent	Lateral	Normal	15	8.1	1.2	7	255	1.835	+	-	-	-	-	-	-	+	.
40	343/24	Hanging	12	Case	43	Male	Primary or below	Hindu	Non-skilled	Y	Y	Y	Y	Y	N	N	N	N	N	N	+	+	+	Cotton	Hard	Complete	Grooved	Posterior	Congested	17	1	1.1	6.1	5	0.47	+	+	-	+	-	-	-	-	Chronic
41	359/24	Hanging	6	Case	33	Female	Higher studies	Hindu	Skilled	Y	N	N	N	N	N	N	N	N	N	N	+	+	-	Cotton	Soft	Partial	Prominent	Posterior	Congested	12	3	1.2	8.5		1.02	+	-	-	+	-	-	-	+	.
42	391/24	Hanging	6	Case	14	Female	Highschool	Hindu	Student	N	N	N	N	N	N	N	N	N	N	N	+	+	+	Synthetic	Soft	Complete	Prominent	Posterior	Congested	9	0.9	1.2	4.9	174	1.5	+	+	-	+	-	-	-	-	Chronic
43	394/24	Multiple injuries	6	Control	40	Female	Primary or below	Hindu	Non-skilled	Y	N	N	Y	N	N	N	N	N	N	N	-	-	-						Pale	11	0.4	0.9	3.6	435	0.67									
44	396/24	Multiple injuries	6	Control	13	Female	Highschool	Hindu	Student	N	N	N	N	N	N	N	N	N	N	N	-	-	-						Normal	14	0.5	1.8	6.1	265	1.15									
45	397/24	Multiple injuries	6	Control	30	Female	Highschool	Hindu	Housewife	Y	N	N	N	N	N	N	N	N	N	N	-	-	-						Normal	13	3.2	1.1	5.9	256	2.38									
46	398/24	Multiple injuries	6	Control	16	Male	Highschool	Hindu	Student	N	N	N	N	N	N	N	N	N	N	N	-	-	-						Pale	9	0.6	1.1	6.4		2.03									
47	425/24	Poisoning	6	Control	56	Male	Primary or below	Hindu	Non-skilled	Y	N	Y	Y	N	N	Y	N	N	N	N	-	-	+						Pale	13	0.3	2.2	0.9	13	0.31									
48	426/24	Multiple injuries	6	Control	29	Male	Highschool	Non-Hindu	Non-skilled	Y	Y	Y	Y	N	N	N	N	N	N	N	-	-	-						Normal	11	1.4	1.4	9.7	227	0.61									
49	428/24	Poisoning	12	Control	35	Male	Highschool	Hindu	Non-skilled	Y	N	Y	Y	N	N	N	N	N	N	N	+	+	+						Normal	15	0.5	6.2	5.1	27.3	3.149									
50	429/24	Multiple injuries	12	Control	50	Male	Primary or below	Hindu	Non-skilled	Y	N	N	Y	N	N	Y	N	N	N	N	-	-	-						Normal	17	0.6	1.2	5.1	104	0.47									
51	430/24	Poisoning	6	Control	21	Male	Higher studies	Hindu	Student	N	N	N	N	N	N	N	N	N	N	N	-	-	-						Normal	18	1.1	0.9	4.4	14.4	0.86									
52	431/24	Head Injury	12	Control	33	Male	Higher studies	Hindu	Skilled	Y	Y	Y	Y	N	N	N	N	N	N	N	-	+	-						Normal	16	1.4	1	6.1	169	2.43									
53	412/24	Kept open	12	Control	20	Male	Higher studies	Hindu	Student	N	N	N	N	N	N	N	N	N	N	Y	+	+	-						Normal	16	1.1	0.9	6.4	20.8	8.85									
54	387/24	Burn Injury	6	Control	19	Male	Highschool	Non-Hindu	Non-skilled	N	N	N	N	N	Y	N	N	N	N	N	-	-	+						Normal	8	1.2	2.1	7.7	5.94	11.66									
55	364/24	Multiple injuries	12	Control	17	Male	Highschool	Hindu	Non-skilled	N	N	N	Y	N	N	N	N	N	N	N	-	-	+						Normal	15	0.4	1.4	5	216	0.57									
56	352/24	Multiple injuries	12	Control	17	Male	Secondary	Hindu	Student	N	N	N	N	N	N	N	N	N	N	N	-	+	-						Normal	9	0.5	1.8	6.1	215	1.15									
57	393/24	Multiple injuries	6	Control	55	Female	Primary or below	Hindu	Housewife	Y	N	N	Y	N	N	Y	N	N	N	N	-	-	-						Normal	9	0.4	0.8	3.5	129	0.4									
58	347/24	Multiple injuries	12	Control	22	Female	Highschool	Hindu	Housewife	Y	N	N	N	N	N	N	N	N	N	N	-	+	-						Normal	13	0.8	1.2	6.8	19	3.43									
59	320/24	Cardiac Pathology	12	Control	29	Female	Highschool	Non-Hindu	Housewife	Y	N	N	N	N	N	N	N	N	N	N	-	-	-						Normal	14	0.3	0.6	2	263	5.15									
60	287/24	Poisoning	6	Control	23	Female	Secondary	Hindu	Student	N	N	N	N	N	N	N	N	N	N	N	-	-	-						Pale	11	1	5.5	9.5	291	13.77									
61	409/24	Hanging	6	Case	35	Female	Highschool	Hindu	Skilled	Y	N	N	N	N	Y	N	N	N	Y	N	+	-	-	Synthetic	Soft	Complete	Prominent	Posterior	Congested	10	0.4	1.6	4.9	197	0.51	+	+	-	+	-	-	-	-	.
62	468/24	Burn Injury	12	Control	45	Female	Primary or below	Hindu	Non-skilled	Y	N	N	Y	N	N	N	N	N	N	N	-	-	+						Normal	14	0.6	0.7	2.9	220	0.86									
63	419/24	Liver Pathology	12	Control	44	Male	Primary or below	Hindu	Non-skilled	Y	Y	Y	Y	N	N	Y	N	N	N	Y	-	-	-						Normal	18	0.6	1	5.2	132	5.42									
64	418/24	Cardiac Pathology	12	Control	38	Male	Highschool	Hindu	Non-skilled	Y	N	N	Y	N	N	N	N	N	N	N	+	-	+						Normal	16	0.3	0.9	3.6	168	0.67									
65	413/24	Electrocution	12	Control	28	Male	Primary or below	Hindu	Non-skilled	Y	N	N	Y	N	N	N	N	N	N	N	-	+	+						Normal	14		1.1		215										
66	414/24	Cardiac Pathology	6	Control	24	Male	Secondary	Hindu	Non-skilled	N	Y	Y	Y	N	N	N	N	N	N	N	+	-	-						Normal	18		1.1		453										
67	459/24	Head Injury	6	Control	21	Male	Higher studies	Hindu	Student	N	Y	N	N	N	N	N	N	N	N	N	-	-	-						Normal	20	0.5	1.1	5.1	419	0.57									
68	404/24	Head Injury	24	Control	33	Male	Highschool	Hindu	Non-skilled	Y	N	Y	Y	N	N	N	N	N	N	N	-	-	-						Normal	15	0.7	3.9	8.3	130	8.91									
69	402/24	Burn Injury	6	Control	48	Male	Highschool	Hindu	Non-skilled	Y	N	N	Y	Y	N	N	N	N	N	N	-	+	-						Normal	17	0.3	0.9	4.1	359	1.78									
70	399/24	Head Injury	6	Control	49	Male	Primary or below	Hindu	Non-skilled	Y	Y	Y	Y	N	N	N	N	N	N	N	-	-	-						Normal	16	1	5.1	9.5	288	13.77									
71	388/24	Lung pathology	6	Control	27	Male	Primary or below	Hindu	Non-skilled	N	N	N	Y	N	N	N	N	N	N	N	-	+	-						Normal	17	1.4	1.5	7.7	402	14.28									
72	378/24	Multiple injuries	6	Control	35	Male	Highschool	Hindu	Non-skilled	Y	Y	Y	Y	N	N	N	N	N	N	N	-	-	-						Pale	16	0.6	1.2	6.3	236	0.79									
73	380/24	Head Injury	6	Control	31	Male	Secondary	Hindu	Non-skilled	Y	N	Y	Y	N	N	N	N	N	N	N	-	+	-						Normal	14	1	2.7	10	66.3	0.71									
74	500/24	Brain Pathology	6	Control	43	Male	Primary or below	Hindu	Non-skilled	Y	N	N	N	N	N	N	N	N	N	N	+	+	+						Normal	19	0.6	1.3	5.9	53.5	2.57									
75	502/24	Head Injury	12	Control	21	Male	Highschool	Hindu	Non-skilled	Y	N	N	N	N	N	N	N	N	N	N	-	+	+						Normal	17	0.6	1.7	5.9	61.7	2.57									
76	446/24	Snake bite	6	Control	43	Female	Primary or below	Hindu	Housewife	Y	N	N	Y	N	N	N	N	N	N	N	-	-	-						Pale	13	1.1	1.6	7.3	30.4	4.2									